THE POPULATION GENETICS OF THE HUNTSMAN SPIDER *DELENA CANCERIDES* (SPARASSIDAE : ARACHNIDA)

A thesis submitted for the degree of Doctor of Philosophy of the Australian National University

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POSTSCRIPT

Following writing, a computer model of the hypothesised AA/BB hybrid zone was successfully constructed. In successive simulations over 50 generations, the majority of males on the AA side of the hybrid zone were consistently of the $A^{ax}B$ type, as predicted in Chapter 6. Furthermore, this occurred without the necessity of attributing any selective advantage to individuals with a hybrid ancestry. This work is currently in preparation for publication.

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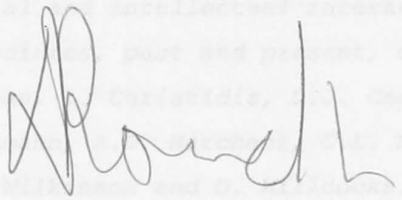
DECLARATION

The research carried out in the course of this investigation and the results presented in this thesis are, except where acknowledged, the original work of the author.



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ABSTRACT

Social behaviour in spiders is a rare phenomenon, and the presence of social behaviour in the huntsman species *Delena cancerides* is particularly remarkable because, unlike the majority of social spider species, *D. cancerides* is a hunting rather than a snare building species.

This work presents an analysis of the evolution and population genetics of *D. cancerides* using cytological and electrophoretic techniques. In contrast to other spider species reported in the literature and examined here, *D. cancerides* shows considerable regional variation in karyotype morphology and meiotic behaviour. Of particular interest is the observation that three populations exhibit permanent chromosomal heterozygosity in males, but homozygosity in females. Previous suggestions that chromosomal systems of this kind may play a role in the evolution and maintenance of social behaviour are discussed, along with three alternative models for the origin of this chromosomal system.

From an analysis of chiasma position and electrophoretic variation it is concluded that the genetic effects of permanent sex-linked chromosomal heterozygosity in *D. cancerides* are negligible, and that its presence is not related to social behaviour in this species.

Of the three models presented for the origin of this system, a hybrid origin is considered most probable. This conclusion is based on the larger allele number in two of the complex heterozygote races, the existence of races with the same chromosomal morphology as the hypothesised parental types, and the fact that this model does not require that sex-linked chromosomal heterozygosity confer any adaptive advantage. The model proposes that the genetic ability to segregate chromosome multiples successfully was central to the survival of the hybrid races. Such a strategy represents an alternative to the development of reproductive isolation (and hence speciation) as an escape from the disruptive effects on fertility which generally result from hybridisation between groups with monobrachial homology.

CHAPTER 1.

BACKGROUND, LITERATURE REVIEW AND AIMS.

1.1 INTRODUCTION

Spiders are an ancient group with an ancestry even older than the Insecta. Indeed, on the basis of distributional data, it appears that many of the older families were present in the early Mesozoic era (Main 1981). Consequently, there has been a remarkable ecological and behavioural radiation, with spiders inhabiting many ecological niches on all continents. Surprisingly, despite the conspicuousness of this group, no comprehensive studies on the population genetics of any spider species have been published.

Approximately 300 spider species have been analysed cytologically, but most of this work was carried out in the 1940's and 1950's before the advent of modern chromosomal banding techniques, and consisted mainly of descriptions of chromosomal morphology and number (see, for example, Suzuki 1950). A notable exception to this is the work of Revell (1947), which contains a detailed description of X-chromosome segregation behaviour in the genus *Tegeneria*. More recently, Maddison (1982) reported on variation in chromosomal rearrangements among species of the jumping spider genus *Pellenes*.

Protein electrophoresis was first used on spiders by Pennington (1979) to investigate a taxonomic problem in the genus *Meta*. On the basis of fixed differences and the deviation of some variable loci from Hardy-Weinberg equilibrium he was able to distinguish between the morphologically identical juveniles of two species. Since this time, larger population studies have been carried out on three species of cave spiders of the genus *Nesticus* (Cesaroni *et al.* 1981) and on two

species of social spiders, *Anelosimus eximius* (Smith 1986) and *Acharanea wau* (Lubin and Crozier 1985). In the social species, conclusions were drawn regarding dispersal patterns and colony structure, but because of the unusually low polymorphism levels encountered in both species, these were equivocal. Electrophoretic data on non-social, surface dwelling spider species are scant.

This thesis deals with an extensive study on the population genetics of the social Australian huntsman spider *Delena cancerides*. As part of this study, electrophoretic and chromosomal data from this species will be compared with those of related non-social genera and other social spider species, with the aim of examining one of the basic assumptions of sociobiology - that is, that the evolution of social behaviour has, of necessity, a basis in kinship genetics.

1.2 THE FAMILY SPARASSIDAE

1.2.1 The taxonomic status of the Sparassidae.

The status of the Sparassidae, of which *D. cancerides* is a member, is discussed in Platnick and Levi (1973) and Croeser (1986), and summarised below.

The name Sparassidae was first used by Bertkau in 1872. The type genus *Sparassus*, however, has no certain type species, and so the status of the family name Sparassidae remains dubious. Heteropodidae (Thorell 1873) and Eusparassidae (Jarvi 1912) were erected in an attempt to resolve this problem. Thus Heteropodidae has priority over Eusparassidae if the type for *Sparassus* cannot be identified. As there has been no recent comprehensive revision of this family, the name Sparassidae will be used here. Sparassidae is also the name used most commonly for the family in the literature (Croeser 1986).

1.2.2 Diagnostic features.

Irrespective of the precise designation used for this group, it is well defined on the basis of a number of diagnostic characters. Huntsmen are generally large spiders, with a flattened body and laterigrade legs - presumably an adaptation for living under bark and in crevices. They have a cheliceral boss, two pectinated claws on each leg, scopulae on the metatarsi and tarsi, a colulus, and eight eyes in two transverse rows of four. In many Australian species the palpal embolus is coiled up to twelve times around a grooved, drum-shaped projection.

1.2.3 Species and distribution.

The Sparassidae comprises 61 genera, containing 818 species (Roewer 1954, Brignoli 1983). These are distributed mainly in tropical and temperate areas, representatives being absent or rare in Europe, Russia, North America, New Zealand and the Arctic and Antarctic circles. The richest fauna is found in Africa, South America, Asia and Australia. In Australia, Roewer (1954) recognises 93 species belonging to 10 genera, of which four of the latter are endemic or have a distribution centred on Australia (Table 1.1).

Specimens most commonly collected in Australia belong to the genera *Olios, Isopoda, Delena,* and *Heteropoda*. *Heteropoda* is restricted to the northern half of Australia while the other three genera are distributed throughout the continent.

The two most likely routes for the entrance of Sparassids into Australia are through Asia, or from the south prior to the break-up of Gondwanaland. The northern route is favoured for two reasons:

(i) cool beech forests predominated on the southern route through Antarctica (Archer 1984). Huntsman spiders are most common in tropical and temperate areas, and beech trees do not provide

large bark exfoliations for shelter. These forests were continuous with those occurring in New Zealand today, but the family Sparassidae is absent from New Zealand except for recent introductions from Australia (Forster 1970).

(ii) huntsmen are most common and speciose in northern Australia, and genera such as *Heteropoda* are also present in New Guinea and Asia.

Endemic genera such as *Zachria* and *Delena* and genera with distributions centred on Australia most probably arose and radiated within Australia, and so the locations of the most 'primitive' populations are not necessarily in the north. No genetically or morphologically based phylogenetic studies have been carried out on these genera however, and so no data are available with which to examine this.

1.2.4 Sister group.

The family Selenopidae appears to be the most closely related group to the Sparassidae in Australia (R.J. Moran, R. Raven pers. comm.). This family is represented by a single species in Australia, *Selenops australiensis*. Selenopids are superficially similar to sparassids, but lack scopulae and possess six eyes in the anterior row and two in the posterior row.

1.3 THE GENUS *DELENA*.

1.3.1 Taxonomy

Roewer (1954) lists two species in this sparassid genus - *D. cancerides* and *D. craboides*, however it may well be that these two species are synonymous. From Walckenaer's original description of *D.*

craboides (Walckenaer 1837), this species is indistinguishable from *D. cancerides* and the type locality is vague - "Australia or New Holland". Furthermore, the type for *D. craboides* cannot be found (Simon 1880), and it is possible that Walckenaer may have described the same specimen twice, under two different specific names.

A number of other species originally placed in this genus by Walckenaer and others have since been moved to other well-defined sparassid genera (*Zachria* and *Isopoda*), and in one case, to a different family.

1.3.2 Diagnostic features.

D. cancerides is easily distinguished from other sparassid species on morphological grounds, and by its unique social behaviour. Because their is no mention of the social behaviour of *D. cancerides* in the literature, it will be described in detail later (Chapter 3).

D. cancerides is a very large species with adult female body lengths often in excess of 30mm, and sometimes up to 45mm. The flattened body and laterigrade legs common to the sparassids are more marked in this species than in any other. This is so extreme that, when at rest, the full length of the legs is in contact with the substrate. The adult coloration ranges from tan to orange, in contrast to the usual brown or grey of other large sparassids, and the anterior median eyes are much closer to each other than they are to the lateral eyes.

The flattened body and eye distribution are sufficient to distinguish *D. cancerides* from all other sparassid species.

1.3.3 Distribution.

D. cancerides has been found wherever major collections have been made in Australia and its associated islands (Hogg 1902). The preferred habitats and life history will be discussed in detail in Chapter 3.

1.4 THE CHROMOSOMES OF SPIDERS

The majority of karyotypic studies on spiders were carried out in the 1940's and 1950's (see Figure 1.1 for refs). Because these studies pre-dated the discovery of modern chromosomal banding techniques, most took the form of comparative analyses of chromosomal morphology and number between species, and observations of male meiosis.

Data now exist on 296 species from 28 families, which, with the exception of three species, all belong to the infraorder Araneomorphae ('true spiders') - see Platnick and Gertsch (1976) for a discussion of the taxonomic basis for these classifications. Of these three, *Heptathela kimurai* belongs to an ancient lineage, the Liphistiomorphae (Mesothelae), which is morphologically distinct from all other suborders of spiders, and carries many primitive features. The other two species, *Atypus karschi* and *Dugiesiella hentzi* belong to the infraorder Mygalomorphae which appears to have a much more recent common ancestor with the araneomorphs (Platnick and Gertsch 1976).

Spider karyotypes are notable for their uniformity in chromosome morphology within and between species, and for the presence of multiple X-chromosomes.

1.4.1 Chromosomal morphology.

With the exception of some members of the families Segestridae and Dysderidae which have holocentric chromosomes (Benavente and Wettstein 1980), the chromosomes of all of the spider species that have been karyotyped to date have localised centromeres. Furthermore, within a species the chromosomes are usually of uniform size and are telocentric. Consequently, only rarely is it possible to distinguish homologous pairs in mitotic preparations. At meiosis, monochiasmate bivalents are generally formed, but in some cases two chiasmata

occasionally occur (Maddison 1982). The majority of chiasmata are terminal, although interstitial chiasmata may also occur. Because the chromosomes are telocentric, it is generally not possible to determine whether the terminal crossovers are proximal or distal to the centromere from the karyotypes that have been published. In *Tegenaria domestica* one of Revell's (1947) figures indicates that the majority of the terminal chiasmata may be distal, however in an unidentified species of *Lycosa*, the only spider species from which C-band data has been published, the majority are clearly proximal (Brum-Zorilla and Postiglioni 1980). In some species of the genus *Pellenes* the chiasmata are generally distal, while in others they are mainly proximal (Maddison 1982).

Given the nature of the spider karyotype, and the absence of polytene chromosomes, rearrangements such as paracentric inversions cannot be detected in gross stained preparations. Fusions and translocations are rare in spiders and have been recorded in only ten of the three hundred species examined. With one exception, the rearrangements in these ten cases have taken the form of multiple centric fusions. Consequently the total chromosome arm count or fundamental number (FN) is the same in these species as in their closely related telocentric relatives. Table 1.2 shows the karyotype morphology of all spider species known to possess non-telocentric chromosomes. Except for species of the salticid genus *Pellenes*, which will be discussed separately, when autosomal fusions have occurred, all of the autosomes are involved unless, of course, the haploid number is uneven in which case one telocentric pair remains unfused. In *Heteropoda sexpunctata* and one population of *Crossopriza lyoni* the two original telocentric X-chromosomes have also become fused to each other. In *Oxyopes ramosus* and an unidentified *Lycosa* species only the two X-chromosomes are fused, the 20 autosomes remaining telocentric.

Thus it appears that in spider genomes fusion is virtually an "all-or-nothing" phenomenon, but the factors controlling fusion in X-chromosomes and autosomes may be different. When X-chromosomes are involved in centric fusions, they are always fused to the other X's, not to the autosomes. This may be the result of preferential fusion between the X's, or perhaps X-A fusions have been selected against. Thus appears that there may be some mechanism in spiders which, when triggered, causes all of the autosomes, X-chromosomes or both to fuse at the same time or in rapid succession. Less spectacular examples of apparently multiple spontaneous chromosomal rearrangements also exist in other groups (King 1982).

In isolated populations of *Mus musculus* in Europe and Great Britain, successive fusions appear to have arisen and been fixed in the homozygous state, but these populations carry a mixture of telocentrics and fusion metacentrics (Gropp and Winking 1981, Nash *et al.* 1983). Fifty-three different arm combinations have been recorded from feral populations, some of which have clearly arisen more than once (Gropp and Winking 1981, Nash *et al.* 1983). The fusions appear to be random, except that chromosome 19 and the X-chromosome are never involved. These two chromosomes have been involved in spontaneous fusions in laboratory populations however, which suggests that selection does not favour their fixation as arms of metacentric chromosomes, rather than that they are unable to fuse.

Capanna *et al.* (1976) argued that in *Mus*, the fusions arose in the germ line of individual dominant males in small populations, presumably in the heterozygous state (although this is not stated), with subsequent fixation through inbreeding. If this is correct many *de novo* fusions would be lost by chance. If the spider fusions have been fixed in the same way, chromosomal fusion in spiders must be a much more frequent event than in mice. Alternatively, fixation may

have been aided by selection, since generally all of the chromosomes are fused, and the fusions are all in the homozygous state.

One example where chromosome fusion may have an adaptive value involves the dog whelk, *Nucella lapillus* (Bantock and Cockayne 1975, Bantock and Page 1976). In this species, eight pairs of chromosomes are consistently present, but the remainder of the chromosomes may be present as 10 pairs of telocentrics, resulting in an overall chromosome number of 2n=36, or as five pairs of metacentrics, apparently fusions, giving a total diploid number of 26. Furthermore, in some populations both telocentric and acrocentric chromosomes may be present, giving an intermediate chromosome count. In these populations, where heterozygosity for a chromosome fusion occurs, a trivalent is formed at meiosis which apparently segregates alternately, thus ensuring the production of balanced gametes (Staiger 1954).

The 2n=26 form is by far the commonest, being the only form recorded on the eastern seaboard of the United States and in Norway, but it is not possible to determine whether the karyotype possesses *the same* fusions in all localities (Bantock and Cockayne 1975, Bantock and Page 1976). In areas where both karyotypic forms are present, there is a statistically significant correlation between karyotype morphology and degree of exposure and water movement - the 2n=36 form is consistently associated with areas where tidal currents are weak and wave activity low, and forms with intermediate chromosome numbers occupy shores intermediate in exposure (Bantock and Cockayne 1975). This phenomenon is a good example of a possible adaptive value for chromosome fusion, but unfortunately the data remains circumstantial, fragmented and incomplete. Controlled breeding experiments cannot be carried out easily (Bantock and Page 1976), and electrophoretic studies, which could elucidate the phylogenetic affinities of the local populations are nonexistent.

1.4.2 Chromosome number.

Figure 1.1 shows the frequency of diploid autosome numbers for all spider species analysed. Four peaks are evident - at 2n=20,22,26 with a smaller peak at 2n=40. The peaks at 2n=22 and 26 are largely accounted for by the fact that the Argiopidae (2n=22) and the Lycosidae (2n=26) have received a disproportionately large amount of attention from cytologists. When this is taken into account, the peaks at 2n=20 and 2n=40, which each consist of several non-related families, suggest that chromosomal doublings may have occurred in the past, with subsequent slight variations in chromosome number through aneuploidy or else rare chromosomal rearrangements. Ancient changes in ploidy level have also been hypothesised to explain similar patterns in chromosome number in a number of groups of fish (see for example Birstein and Vasiliev 1987). In these cases, however, the argument is strengthened by the fact that there is considerable variation in chromosome morphology within the karyotypes and so the various haploid chromosome sets can be identified.

The chromosomal complement of *Heptathela kimurai* (2n=92) is not consistent with the occurrence of simple doubling, but since it represents a distinct lineage, it may have diverged prior to the origin of the proposed 2n=20 ancestor of the other lineages. Platnick and Gertsch (1976) considered the high chromosome number of *H. kimurai* to be a derived character common to the liphistiids, based on an outgroup comparison with the order Amblypygidi. Given the observed lability of chromosome number within the infraorder Araneomorphae, however, such conclusions based on chromosomal variation between groups as distant as orders should not be taken seriously.

The two mygalomorph species have diploid values of 42 and 44 which is also consistent with a chromosome doubling, from an ancestor with around 20 chromosomes. Thus the common ancestor of the mygalomorphs and the araneomorphs may have had 20 chromosomes.

Suzuki (1954) argued that because of this inverse correlation between chromosome number and degree of specialisation, the primitive chromosome number was high and spider karyotypic evolution has been characterised by a progressive *decrease* in chromosome number. If this were true, species with low chromosome numbers might be expected to carry larger chromosomes. No such relationship exists, however (Suzuki 1954). Furthermore, there is no reason to expect a primitive morphology to necessarily be accompanied by a primitive karyotype.

1.4.3 Sex chromosomes.

Multiple sex chromosomes occur sporadically in a number of animal groups (White 1940). Some 81% of the spider species that have been studied have an X_1X_2(male)/$X_1X_1X_2X_2$(female) sex determining mechanism. That is, males possess one copy of each of the two X-chromosomes while females carry two copies of each. This system is clearly the ancestral state, since its occurrence is widespread throughout all of the major families. Of the remaining 19%, 10% have reverted to the X/XX condition more usual for invertebrates, 8% have an $X_1X_2X_3/X_1X_1X_2X_2X_3X_3$ system, and 1% have an $X_1X_2X_3X_4/X_1X_1X_2X_2X_3X_3X_4X_4$ system. The X-chromosomes are generally indistinguishable from the autosomes at mitosis although in a small number of species one of the X's is consistently larger (Suzuki 1954). In the few species where female meiotic preparations have been obtained, the X's form normal bivalents with their homologue and each contain a single chiasma (Hackman 1948, Suzuki 1954). These show no differential contraction or staining properties when compared to the autosomes, and so cannot generally be distinguished from the autosomal bivalents at female meiosis. In marked contrast to this, the X's condense very early during male meiosis and are visible at leptotene and pachytene, usually as a single amorphous, heavily staining body, although the individual X-

chromosomes can sometimes be distinguished at these stages. By diplotene the individual X's can be distinguished, but still remain closely associated side by side. Despite this close association, no chiasmata are formed and no synaptonemal complex is visible between them (Wise 1983) suggesting that they are non-homologous. The X's always segregate in tandem to the same pole at anaphase I and then dissociate, although the differential staining may persist. The second meiotic division is normal.

Y-chromosomes have not been reported in spiders, but a possible incipient case has been described for a number of species of the genus *Pellenes* (Maddison 1982). This is discussed in detail in section 1.6.2.

1.4.4 Chromosomes in the Sparassidae.

Table 1.3 gives the chromosome numbers of all of the huntsman species which have been karyotyped at the time of writing. Two basic autosome numbers, 2n=38 and 2n=40, occur in this family (Table 1.3), but, as no phylogenetic data exists for these genera, it is not possible to determine whether these represent primitive or derived states, two distinct lineages, or multiple convergences.

All of the sex chromosome numbers known to occur in spiders (from one to four) are represented in this family, but since three X-chromosomes occur in six of the seven genera, and in both the 2n=40 and 2n=38 groups, this probably represents the primitive condition.

The karyotype of *Heteropoda sexpunctata* (2n=21) has been derived by multiple fusions from a 2n=38+X_1X_2 ancestor, since the autosomal arm number is 38, and the single X-chromosome is metacentric.

1.5 SOCIAL BEHAVIOUR IN SPIDERS

Social behaviour in the Isoptera and the Hymenoptera has been the subject of a large amount of work, perhaps because of the presence of sterile castes. This is because the existence of nonreproductive workers in these groups apparently contradicts classical evolutionary theory which states that an animal's fitness is measured by its ability to reproduce. Social behaviour in spiders on the other hand has, until recently, received little attention because it appears to be less highly evolved, and evidence for sterile workers is in this group is scant and purely circumstantial.

Burgess (1978) defined two criteria for diagnosing social behaviour in spiders:

(i) the species must be found in statistically demonstrable clumps, and

(ii) individuals must exhibit some form of communication or interaction beyond that seen in male/female pairs.

On the basis of these two criteria Burgess recognised 33 social species among the 30,000 spider species that have been described. Since 1978, social behaviour has been reported in two more species, both Australian (Rowell 1985, Main 1987). In these 35 species, social organisation ranges from loose aggregations of conspecifics to close kin groups cooperating in prey capture, communal feeding and web or nest building.

Social behaviour in spiders has clearly arisen independently on a number of occasions since it occurs sporadically in several unrelated families. Even so, some characteristics are almost universal among these social species:

(i) sex ratio is heavily skewed in favour of females (Vollrath 1986). This is the case in all social spider species where the sex ratio has been analysed. Karyotype analysis of the social thomisid species *Diaea socialis* suggests that this bias is evident at the embryonic stage (unpublished data). Vollrath (1986) claims that this is also the case in *Anelosimus eximius*, but gives no data to support his claim. This sex bias may have been selected because:

(ii) in social spiders males do not generally help in colony maintenance, prey capture, or defence (Vollrath 1986). Vollrath speculates that this may be because males have reduced poison glands, and so are not as well adapted to soldier duties. In the eusocial Hymenoptera it has been argued that males, social or not, rarely provide parental care and so do not possess the necessary biological preconditions for the evolution of social behaviour (Alexander 1974, Craig 1982). The same argument could also be applied to spiders.

(iii) most social spiders are confined to the tropics (Nentwig 1985). There are four exceptions to this, including the two recently reported Australian species. Nentwig (1985) suggests that this may be a result of increased prey availability, however it may also be a function of increased species numbers in the tropics compared to temperate regions.

(iv) in social spiders, a web is most commonly used to catch prey and for communication (Burgess 1978, Nentwig 1985, Darchen

and Delage-Darchen 1986). Buskirk (1981) believes the ability to build a web has been a major preadaption to the evolution of sociality in spiders, as it serves as a focus for colony activity, a means of communication, and an effective device for prey capture. There are two exceptions to this rule however; the Australian social species *Delena cancerides* and *Diaea socialis*. In *Diaea socialis*, silk is used for nest construction (Main 1987), but neither species builds a web snare.

(v) clutch size in social spiders is often lower than in non-social relatives (Buskirk 1981, Nentwig 1985). This may reflect a lower juvenile mortality rate in social species.

(vi) larger prey is captured than by closely related or similar sized solitary species (Burgess 1978, Nentwig 1985). This is clearly made possible by cooperative prey capture, however it has not been demonstrated whether this results in more food per individual. Buskirk (1981) has pointed out, however, that larger arthropod prey items have proportionately less cuticle (which is not eaten) than do smaller individuals, and hence their food value per total weight is greater.

(vii) finally, in social spiders, colony structure is open (Darchen and Delage-Darchen 1986). That is, unlike the eusocial insects, unrelated conspecifics are tolerated by colony members. This is one of the most striking features of social spiders; whereas most spiders are fiercely aggressive and cannibalistic, social species tolerate the presence of

conspecifics, and even commensals such as beetles and other spider species (Buskirk 1981). In solitary species, female spiders usually care for their egg sac. Newly hatched spiderlings remain close to the mother and are sometimes even fed by regurgitation. This may represent a preadaptation to the evolution of social behaviour in this group, since it is similar, but less extreme, to the tolerance seen in social species (Buskirk 1981). Burgess (1978) suggested that the tolerance observed in social species may have arisen through neoteny, such that the tolerance between hatchling sibs and the mother's tolerance of her offspring is maintained indefinitely. While this tolerance of conspecifics is generally the case in social spiders (Darchen and Delage-Darchen 1986), in the social spider *Agelena consociata* injured members, or individuals whose smell has been changed with ether or ethanol, are attacked and killed (Burgess 1976).

Burgess (1979) listed the main advantages to be gained from social behaviour as follows:

(i) increased shelter,

(ii) better chances of finding a mate,

(iii) greater area of the catching web,

(iv) more food available for young and gravid females (but see vi above).

No spider species have been reported with morphologically distinguishable castes as is the case in the eusocial Hymenoptera and Isoptera. This may stem from the fact that spiders do not require any complex brood care (Burgess 1978), and because the aggressive behaviour and poisonous bite necessary for prey capture make all of

the spiders in a colony effective 'soldiers' anyway. A dominance hierarchy, not necessarily correlated with size, has been demonstrated in the social spider *Agelena consociata* however (Buskirk 1981), and there is evidence that the social spider species *Anelosimus eximius* has nonreproductive 'helpers' (Vollrath 1986). Vollrath found an average sex ratio (male:female) of 0.15 and, on average, within colonies there were 17 times more adult females than eggsacs - that is, not all adult females are reproducing at any one time. Furthermore, in a sample of 68 adult females 40 had not been fertilised, and this group included young and old females. Since the average female lifespan as an adult is only 76 days (Aviles 1986) whereas sperm can be stored for long periods (Vollrath 1986), it is likely that these old females were never inseminated and would die without reproducing. Thus Vollrath concluded that "females of *A. eximius* can be divided into those which lay eggs and those which do not". Since all of the females possessed semen receptacles and showed no morphological divergence, it is probable that the non-inseminated females were facultative rather than obligate nonreproductives.

Extreme monomorphism has been observed in all of the social spider species that have been analysed electrophoretically. In order to investigate dispersal patterns, Lubin and Crozier (1985) examined 29 enzyme loci in eight populations of the social spider *Acharanea* wau, and found polymorphism at only one locus, αGPD. Moreover, only one of the eight populations analysed actually carried the polymorphism, otherwise each population was completely homozygous for one or other of the two alleles detected. These data were consistent with the initial predictions of these authors, that individuals in a colony are closely related, and that local populations are derived from a single parent colony. However, this study was based on only one variable locus, and additional work is required to confirm their observations.

Two methods of colony foundation occur in the social species *Anelosimus eximius;* budding, and dispersal of individual females who may subsequently re-aggregate at another site with other dispersing females. Smith (1986) postulated that the former would be likely to result in inbreeding, the consequent subdivision of populations into isolated genetic lineages, and hence high levels of population differentiation. Thus high genetic similarity within colonies and colony clusters, accompanied by differentiation between geographically isolated populations would be expected. In order to test this prediction, 187 animals from 23 colony clusters collected from two geographic sites, in Panama and Suriname, were analysed electrophoretically at 51 loci. Very high levels of monomorphism were found, with only seven of the 51 loci demonstrating polymorphism. Two of these seven loci were monomorphic within, but differed between, the two geographic areas. When these populations were examined separately, polymorphism was evident at only three (Suriname) and two (Panama) of the 51 loci. Only two clusters showed polymorphism, in each case for a single locus, and in both no deviation from Hardy-Weinberg equilibrium was observed. Smith compared unpublished data on polymorphism levels of solitary spiders with those of *A. eximius* and *A. wau* and noted that the levels were lower in the social species. This suggests that low polymorphism and social behaviour may be in some way connected, perhaps as a result of inbreeding. Smith suggested that the genetic uniformity within colony clusters may indicate that colony clusters result from the budding off of a single colony, rather than "aggregations of unrelated webs at particularly favourable sites."

Table 1.4 shows the percentage of polymorphic loci and average heterozygosity per locus per individual for the spider species for which data are available. The two social species have the lowest polymorphism levels and the lowest heterozygosity level is also found in a social spider.

1.6 CHROMOSOMAL TRANSLOCATION HETEROZYGOSITY

Genes carried on the same chromosome are linked in inheritance. Assuming random chiasmata distribution, the degree of linkage, that is the degree to which they are inherited together, will depend upon their distance apart on the chromosome. Genes which are close together will rarely recombine, whereas genes which are far apart on their chromosome will regularly recombine at meiosis. In the absence of recombination, as in achiasmatic meiosis, or with recombination restricted to localised regions either proximal or distal to the centromere, linkage will be virtually absolute. In most organisms, however, linkage is only partial; chiasma position may vary, and genes carried on different chromosomes assort independently of one another at meiosis unless there is a non-random segregation of specific chromosomes.

Chromosomal interchanges and fusions may alter both linkage and random assortment when present in the heterozygous condition, because such rearrangements extend linkage beyond the limits of a single chromosome, and require preferential segregation for the production of genetically balanced gametes.

In such cases (Fig. 1.2) the chromosomal linkage arises as a result of heterozygosity for the chromosomal fusion (I) or translocation (II), and consequently this linkage will not be maintained in the next generation if one of the gametes fuses with another of the same type, since this produces a zygote homozygous for either the rearranged or the ancestral chromosomes. If two conditions are met, however, it is possible for heterozygosity for these arrangements (and hence linkage) to be maintained in future generations *ad infinitum*. Firstly, alternate segregation must take place consistently and, secondly, the zygotes that form must originate from gametes carrying complementary chromosomal types.

1.6.1 Permanent translocation heterozygosity: *Oenothera* and *Isotoma*.

A classic example of chromosomal linkage via permanent translocation heterozygosity occurs in the plant genus *Oenothera* (2n=14). This case has been reviewed by Cleland (1972). Some populations in this genus are heterozygous for only a small number of translocations, but in others many, and in some cases all, of the chromosome pairs have been involved in reciprocal translocations. Meiotic behaviour in these species ensures that unbalanced gametes are not generated. This occurs because, rather than forming independent bivalents, meiotic pairing leads to the formation of large ring or chain multiples in which the translocated chromosomes always appear in the same order. Segregation of these chromosomes at meiosis is thus no longer independent. Rather, alternate chromosomes in the ring or chain migrate to the same spindle pole during the first division of meiosis, the remaining chromosomes moving to the opposite pole. Thus, despite the fact that each chromosome is a discrete physical entity, every gamete within a particular species invariably carries one of two groups of chromosomes. In the genus *Oenothera* three major genomic complexes can be recognised on the basis of their morphological effects (Stubbe and Raven 1979). This chromosomal linkage is maintained by two additional mechanisms that ensure perpetual heterozygosity for the translocations. These involve either balanced lethal alleles, whereby neither chromosome group can produce viable zygotes in the homozygous state, or else gametic lethals, which determine that pollen can only fertilise ova containing the opposite chromosome type.

A second extensively studied plant species, *Isotoma petraea* (2n=14) from Western Australia, possesses a similar chromosomal system, with the number of translocations usually consistent within, but differing between geographically isolated populations (James

1965). In the region occupied by the multiple interchange populations, there is a rough cline in ring size from rings of four chromosomes in the extreme north-east, to fourteen in the south-west.

In both *Oenothera* and *Isotoma*, normal diploid populations routinely outbreed, while the translocation races are obligate inbreeders. Their origin may have involved an initial hybridisation event between normal bivalent-forming populations homozygous for different sets of translocations, resulting in heterozygous populations (Cleland 1972). Alternatively, the incorporation of chromosomes into the ring may have been effected in a stepwise fashion by the fixation of new translocations in the heterozygous state, perhaps accelerated by founder effects (Darlington 1931, James 1965,1970). These two mechanisms are not, of course, mutually exclusive. Some species of *Oenothera* may share one of their complexes indicating that hybridisation has occurred, but whether this has been the major or sole mechanism in the development of translocation heterozygosity in this group remains unclear.Protein electrophoresis h as demonstrated that complex translocation heterozygosity in these groups can produce stable genic heterozygosity through the isolation of different alleles on each complex. In *Isotoma*, James *et al.* (1983) discovered fixed heterozygosity at some loci in ring-carrying populations, and the overall heterozygosity levels were 12.5 times higher in these populations than in the chromosomal homozygotes. However, no locus was consistently heterozygous in all populations with translocation heterozygosity. Levy and Levin (1975) showed fixed heterozygosity at a small number of loci in some populations of ring-forming *Oenothera* species but again this was not consistent between populations, and they concluded that "the ability to accumulate and/or retain heterozygosity and variability has not been accompanied by extraordinary levels of either." Thus, whether the maintenance of

genic heterozygosity via this chromosomal system represents an adaptive strategy or selection is simply capitalising on a chance phenomenon in these groups remains unclear.

1.6.2 Permanent Sex-Linked Translocation Heterozygosity in Animals.

Permanent translocation heterozygosity of the kind found in *Oenothera* and *Isotoma* has not been reported in animals. However, translocations involving the sex chromosomes can result in a similar phenomenon, though one which is restricted to the heterogametic, generally male, sex.

If, in a male organism, part of an autosome is translocated onto an X-chromosome to give rise to a "neo-X" product, the homologue of that autosome can be regarded as a "neo-Y" chromosome, since it must segregate from the neo-X chromosome at the first meiotic division if balanced gametes are to be produced. Similarly, the remainder of the autosome from which the translocation has been derived also becomes a neo-X chromosome, since it must migrate with the X-A translocation product. Consequently, sperm will be of two kinds; one kind carrying the two neo-X chromosomes (that is the X-A translocation product and the remainder of the translocated autosome), and the other carrying the neo-Y (autosomal homologue), which will appear only in male offspring. Conversely, if a translocation occurs between an autosome and a Y or neo-Y chromosome, its non-translocated homologue will move with the X-chromosome and so become, from a segregational point of view, an additional neo-X chromosome. At meiosis, the pairing of neo-X and neo-Y chromosomes gives rise to translocation rings or, more often, chains (Fig. 1.3). Under a translocation system of this kind, males will be perpetually heterozygous for the translocations since they are heterozygous for their sex chromosomes. Females, on the other hand, will be perpetually homozygous for the translocations

involved since they are homozygous for their sex chromosomes. These "neo" sex chromosomes generally differ from the original sex chromosomes in that chiasma formation still occurs, so that there is some degree of recombination between them, and that the organism remains diploid for the material carried on the original autosomes despite its linkage to the sex-determining system. Moreover, the autosomal material generally continues to behave in the same way as the original autosomes in terms of its contraction and staining characteristics at meiosis.

Simple sex-linked translocation heterozygosity of this kind, involving only three or four chromosomes has been reported in males of a number of species (Table 1.5). These small rings and chains have generally been produced by the fixation of only one or two rearrangements.

In species of the jumping spider genus *Pellenes* (Maddison 1982), an autosome has undergone a tandem fusion with one of the two X-chromosomes, while the homologue of the autosome has fused centrically to another autosome. This results in the formation of a multiple-of-three complex at meiosis made up of the autosomal fusion product (A-A), the X-autosome fusion product (X-A) and the unfused autosome, and an associated X-chromosome. Segregation results in the X, X-A and the autosome migrating together to become incorporated into female-determining sperm, and the A-A being incorporated into male-determining sperm. Thus, in terms of segregation, the autosomes which have been involved in fusions behave as X and Y chromosomes, since females are homozygous for the fusions and males are heterozygous. Although Maddison calls this an "XXXY" sex-determining system, the new 'pseudo' sex chromosomes differ from normal sex chromosomes in two respects:

(i) there is chiasma formation between the pseudo X- and Y-chromosomes, and

(ii) both sexes remain diploid for all of the material carried on these chromosomes.

Moreover, the two true X's show different condensation and staining properties compared with the autosomes, while the pseudo sex chromosomes resemble the other autosomes in this respect.

To briefly digress, the situation in *Pellenes* is also of interest because it is the only known spider group where autosomal fusion has occurred only once and the karyotype has not progressed to fusion saturation. Moreover, given that the specific rearrangements in *Pellenes* are unlikely to have undergone precise reversal, on the basis of their phylogeny it appears that the same rearrangements have occurred at least twice. Thus the fact that this group appears to possess mutational "hotspots" and possesses a type of rearrangement (tandem fusion) that has never been demonstrated before in spiders, its departure from the "all-or-nothing" rule of chromosome fusion in spiders is considered here to be an aberration rather than a challenge to the rule.

In sex-linked translocation heterozygosity, a chain of chromosomes is usually formed at meiosis. More extensive sex-linked translocation heterozygosity resulting in long chromosome chains (Fig. 1.3) has been reported on several occasions.

In 1954, Ogawa described a sex-linked translocation chain in males of the centipede *Otocryptops sexspinosus* (2n=15), consisting of nine chromosomes, five of which appeared only in males. The other four behaved as X chromosomes and were homozygous in the female. Thus this system behaved as an X_{1-4}, Y_{1-5} sex-determining mechanism. More recently, smaller translocation chains have been discovered in other populations of this species, indicating that the larger chain was probably built up progressively by the gradual incorporation of translocations involving the ancestral X and Y chromosomes or neo-X

and -Y chromosomes (White 1973). This is similar to James' and Darlington's hypothesis for the evolution of translocation heterozygosity in *Oenothera* and *Isotoma* (Darlington 1931, James 1965,1970). *O. rubiginosus*, another member of this genus, also carries chains. In this case the chains consist of five chromosomes, but this condition is not fixed in the population (White 1973).

In the termite *Kalotermes approximatus* (male 2n=32,33), translocation chains also appear to have been built up by the successive fixation of novel rearrangements (Syren and Luykx 1981). The putative ancestral race from southern Florida carries a chain of eleven chromosomes, while populations to the north have chains and rings ranging in size from thirteen to seventeen chromosomes, the number usually being constant within populations. In some cases the fixation of new translocations appears to have led merely to a reordering of the chromosomes in the chains. Since "each of the higher order complexes is found near the next lower one from which it can be derived, in a majority of cases, by a single translocation", Syren and Luykx (1981) outlined two means by which these chromosome races may have evolved:

(i) novel translocations occurred at the periphery of the species range as it advanced northward, and were fixed by drift or selection, resulting in an increase in complex size at the advancing edge of the range, while the ancestral, lower order chains were left in their original ranges.

(ii) the species range remained constant during the evolution of the complexes, which arose by the fixation (as heterozygotes) of rearrangements in small subpopulations within this range, perhaps for some unknown selective advantage they conferred.

Syren and Luykx (1981) concluded that insufficient data was available to choose between two models. However, their suggestion that some selective advantage may be associated with the incorporation of chromosomes into translocation complexes is intriguing.

Geographical variation in chain or ring size also occurs in the related species *Incisitermes schwarzi* (2n=32), where from eleven (Mexico) to eighteen chromosomes (Jamaica) may be involved (Luykx and Syren 1981). The latter represents the longest translocation complex found in any organism to date. Enzyme electrophoretic analysis of a ring-of-fourteen race of this species at one locality showed sex linkage for two loci, acid phosphatase and esterase (Santos and Luykx 1985). At both loci, only one of the two or three alleles present at the given locus is found on the Y-group of chromosomes, while all of the alleles may occur on the X complex. As a consequence, males tend to pass the Y-linked allele to their sons, and whichever allele they carry on the X-complex to their daughters. This linkage is not complete since recombination at meiosis, via chiasma formation, occasionally results in alleles carried on the X complex being transferred to the Y complex. The recombination rates observed were low however (0.3% and 0.6% respectively for the two loci).

The copepod *Mesocyclops edax* (female 2n =14) differs from all other species with sex linked translocation heterozygosity in that no recombination takes place, because meiosis is achiasmate. This species is also unusual because it is polymorphic for two different mieotic configurations present in the same population. In 30% of individuals all fourteen chromosomes are involved in a translocation ring, but in the remainder there is a ring of twelve chromosomes and one free bivalent. It can be deduced from these ring configurations that the two forms cannot be attributed to a single polymorphism for a translocation between one of the bivalent-forming chromosomes and

those in the ring, and it is possible that the two ring types represent sympatric sibling species.

Whether the system described in *M. edax* is sex linked or not is also unclear, because to date only females have been karyotyped. There is evidence to suggest that females are the heterogametic sex in this group (Chinappa and Victor 1979), which is consistent with sex linkage, but the lack of meiotic studies in the male raises the possibility that this system is maintained by balanced lethals as in *Oenothera*, rather than sex linkage. However, in another copepod, *Diaptomus castor*, translocation heterozygosity clearly is sex linked, since chains of six chromosomes occur in females while only bivalents are formed in males (White 1973).

1.6.3 The Biological Significance of Complex Sex-Linked Translocation Heterozygosity.

The evolution and possible functions of translocation heterozygosity in *Oenothera* and *Isotoma* have already been reviewed extensively (James 1965, Cleland 1972), and will not be covered here.

Despite its superficial similarities, sex-linked translocation heterozygosity is very different from the phenomenon observed in *Isotoma* and *Oenothera*, because it is confined to one sex. In this system, the chromosomes segregate randomly in the homozygous sex and hence the X- or Z-linked set undergoes considerable reassortment, while in the heterozygous sex, the Y- or W-linked group of chromosomes remains constant - a unique form of Darlington's "two track heredity" (Darlington 1973).

The following possible consequences of sex-linked translocation heterozygosity have been listed by Syren and Luykx (1981):

(i) restriction of many alleles to males via the Y-complex

(ii) maintenance of extensive genic heterozygosity

(iii) increased genetic similarity between like-sex offspring.

Since the Y-linked group of chromosomes never appears in females and it does not recombine between male lineages, males can evolve, with respect to the genes carried on this group, quite independently of females. Hence differential selection in one sex, or else drift, could result in males becoming ecologically, behaviourally, and morphologically divergent from females, and indeed, from other males. Furthermore, such divergence may be expected to be very rapid, since the Y-linked group never occurs in the homozygous state, and so no reassortment between separate lineages with a consequent dilution of accumulated changes can occur. Thus, a new mutation on the Y-complex of a single male is essentially fixed (albeit in the heterozygous condition) in all of his future offspring. No inbreeding or backcrossing of offspring to the original parent is necessary to prevent loss of the novel mutation from the male lineage.

Surprisingly, increased sexual divergence does not appear to have occurred in termites and, where data from species possessing this chromosomal system are available, the two sexes are remarkably similar. For example *Incisitermes schwarzi*, which carries the longest translocation complexes reported so far, shows no preferential investment of either sex in any of the castes (Luykx *et al.* 1987, Luykx 1986). Colonies involved in nymph and alate formation do show a temporary bias in the sex ratio caused by the earlier development of female workers into nymphs and alates, and their earlier dispersal. Colonies not in stages of nymph or alate formation typically have equal numbers of males and females. Castes made up of only one sex have been reported in *Nasutitermes exitiosis* and some other higher termite species (McMahan 1974), but the longest chains have been reported in lower termites, where this does not occur.

Thus, although there is some electrophoretic evidence for genetic divergence, and consequently the potential for phenotypic divergence,

there is no evidence that the latter has actually occurred. Hence, it appears unlikely that selection for this effect has been the primary cause of the evolution of complex sex-linked translocation heterozygosity in termites.

That the maintenance of genic heterozygosity has constituted a selective pressure for the development of sex linked translocation heterozygosity also seems doubtful, on the grounds of the similarity which exists between the sexes. Although this chromosomal system has been demonstrated to result in the maintenance of genic heterozygosity in males (Santos and Luykx 1985), the apparently identical ecological requirements of the two sexes noted above does not support the argument for differential selection.

From a computer simulation, Charlesworth and Charlesworth (1980) derived a model in which "a necessary condition for selection to favour a fusion between an autosome and a sex chromosome is that the alleles at the autosomal locus are maintained by selection at different frequencies in the two sexes."

As has already been mentioned, in *Incisitermes schwarzi*, which carries the largest translocation complexes known, there are no marked differences between the sexes to indicate differential selection on morphological or behavioural grounds.

While Charlesworth and Charlesworth considered such selection to be "a necessary condition for fusion to be favoured", they emphasised one important qualification:

"This result, of course, depends on the assumptions that the fusions are not themselves associated with any effect on fitness, nor subject to distorted segregation in their favour in heterozygotes."

The possibility that sex linked translocation heterozygosity may indeed have a selective value above and beyond the genotype *per se* has been the subject of discussion, particularly with regard to social

animals (Luykx and Syren 1979, Lacy 1980,1984, Leinaas 1983, Crozier and Luykx 1985, Rowell 1986).

1.6.4 Sex Linked Translocation Heterozygosity and Social Behaviour.

A large number of termite species carry sex linked translocation heterozygosity, and the largest translocation complexes known occur in this group. Some of the twenty-four termite species may share this chromosome system through descent, but the fact that this phenomenon appears sporadically in three separate families (Vincke and Tilquin 1978) indicates that it has arisen more than once in the Isoptera.

Eusocial behaviour which occurs in all termite species, and is characterised by the presence on nonreproductive castes, has also been reported in the Panamanian spider *Anelosimus eximius* (Vollrath 1986). Although no karyotypic data on this species are available, Vollrath has speculated on the possibility that this species may also carry sex-linked translocation heterozygosity, since skewed sex ratios are known to be correlated with this chromosomal phenomenon in mistletoes (Barlow and Weins 1976).

It has been suggested that social behaviour may facilitate the rapid fixation of chromosomal changes, and in particular that inbreeding in social species may increase the chances of fixation of translocation heterozygosity (Syren and Luykx 1977). While many non-social species inbreed, the correlation with social behaviour remains to be accounted for. Furthermore, in some chain-carrying termite species, cross-fertilisation appears to be favoured (Vincke and Tilquin 1978). Thus, given this apparent correlation, it is worth considering the possibility that this chromosomal system has some adaptive function in social species.

As mentioned above, sex-linked translocation heterozygosity can alter the relationships within family groups such that individuals

will be more closely related to their like-sex sibs than to siblings of the opposite sex. The extent of increase in relatedness between like-sex sibs generated by this system is dependent upon the number of chromosomes involved in the translocation complex and the distribution of chiasmata at meiosis. In the most extreme situation where all of the chromosomes are involved, and crossovers are always either distal or else absent, as in *Mesocyclops*, relatedness between like-sex offspring would be 75% compared with 50% between parents and offspring. This is because offspring receive a random 50% of their genes from their mother, but **the same** 50% from their heterozygous father - the Y-linked group in the case of male offspring, and the X-linked group in female offspring. Relatedness between opposite-sex offspring would be 25% through the female parent and zero through the male parent. Although translocation heterozygosity never involves all of the members of the chromosome complement in termites, in two species studied to date a large proportion is involved and the crossovers are generally terminal.

Lacy (1980, 1984) argued that this potential bias in relatedness has played a role in the evolution of eusocial behaviour in the termites, on the same grounds that Hamilton (1964) suggested haplodiploidy has been relevant to the evolution of eusociality in the Hymenoptera. That is, because individuals are more closely related to their sibs than to their own potential offspring, then in terms of passing on their own genes, they will gain more by aiding their parents in the production of more sibs than by reproducing themselves. Moreover, unlike the haplodiploid Hymenoptera, within both sexes more than 50% of their genes are held in common, so both sexes would be expected to participate in worker duties, as is in fact the case. The analogies between the genetic systems of termites and the Hymenoptera are striking, and Lacy's idea has the attraction that it provides a

general explanation for the evolution of eusociality in both groups. Nevertheless, Lacy's hypothesis has been criticised on two grounds:

(i) Although relatedness is enhanced in offspring of the same sex, the corresponding reduction in relatedness between sexes would dictate that workers should preferentially invest in offspring of their own sex. For certain castes, such as soldiers, this is clearly not possible since their labour benefits the colony as a whole (Leinaas 1983). Furthermore, there is no evidence for this idea from data on the spatial distributions of the sexes in a termite colony (Luykx *et al.* 1987).

(ii) Translocation heterozygosity apparently arose in termites **after** the evolution of sterile workers (Crozier and Luykx 1985, Rowell 1986). That this system could have been selected to justify the existence of sterile workers already present is clearly illogical.

Another possible explanation for the apparent selection of this genetic system in termites was put forward by Luykx and Syren (1979) based on the assumption that because there is an increase in the relatedness between same-sex sibs, they will share more genes in common and thus vary less in their genotypes. Consequently, a greater uniformity may be expected in the genetically determined aspects of behaviour, including reactions to different pheromone levels and pheromone release in response to external factors. Clearly uniformity in morphology is not necessarily of great importance since termite colonies owe their efficiency to the range of morphological castes, but some uniformity in communication mechanisms within and between castes, such as pheromone release, may well be advantageous. In a colony whose cohesiveness and competitiveness relies on the reaction

of its members to pheromonal stimuli and behavioural cues, increased uniformity in behaviour and reactions may be expected to result in greater efficiency and ultimately greater reproductive success.

However, there are two major weaknesses in this argument. First, while there is increased uniformity within the sexes, the genetic disparity between the sexes remains, and is in fact increased. This would lead to two uniform groups in a colony rather than one. This problem could be overcome if the two sexes remained relatively separated within the colony, but there is no evidence that this occurs. Secondly, the majority of termite species which carry translocation heterozygosity possess rings of only four chromosomes. Consequently, unless it can be shown that the same chromosomes are consistently incorporated into the ring, any argument for an adaptive function of this system of sex linked translocation heterozygosity is weakened.

Even so, this chromosomal behaviour clearly influences genotype frequency distributions within and between the sexes. It remains to be established whether this correlation with social behaviour is a casual or causal phenomenon.

It could be argued that the correlation between sex-linked translocation heterozygosity and social behaviour is merely an accidental coincidence, due to some peculiarity of the termite karyotype and independent of their social evolution. However, the present work reports on the occurrence of the same chromosomal phenomenon in a social organism from a different class, which provides circumstantial evidence that social behaviour and complex sex-linked translocation heterozygosity may indeed be more than casually linked.

1.7 AIMS

The aims of this study are as follows:

- to investigate the origin and evolution of karyotypic variation in the social huntsman spider *Delena cancerides*.
- to investigate the genetic effects of chromosomal behaviour and social structure in *D. cancerides*, using electrophoretic techniques.
- to determine whether the chromosomal and genetic characteristics of *D. cancerides* have any selective significance both in general and with regard to the unusual social behaviour of this species.

GENUS	NO. OF SPECIES IN AUSTRALIA	OTHER LOCALITIES COLLECTED
Delena	1*	Endemic
Zachria	5	Endemic
Typostola	4	Endemic
Isopoda	39	New Caledonia, New Guinea
Pediana	5	New Guinea, Indonesia
Olios	23	West Indies, Asia, New Guinea, Mexico, USA ,New Caledonia, South America
Heteropoda	9	Asia, Pacific, South America
Pandercertes	2	Asia, Pacific
Anchognatha	1	Pacific

Table 1.1 Distribution of Australian sparassid genera.

* The type for a second species of *Delena, D. crabioides* Walck. 1837, is lost (Simon 1880), and no further specimens of this species have been reported in the literature.

SPECIES	AUTOSOMES		X'S	REFERENCE
Aranea dumetorum	10m	2t	2t	Hackman 1948
Aranea scylla	10m	2t	2t	Suzuki 1951
Dictyna arundinacea	22m	--	2t	Hackman 1948
Dictyna foliicola	22m	--	2t	Suzuki 1954
Heteropoda sexpunctata	18m	2t	1m	Bole-Gowda 1952
Crossopriza lyoni	26m	--	1m	Bole-Gowda 1958
Crossopriza lyoni	22m	--	2t	Sharma et al 1959
Lycosa sp.	20t	--	1m	Postiglioni & Brum-Zorilla 1981
Pholcus crypticolens	22m	--	2t	Suzuki 1954
Oxyopes ramosus	20t	--	1m(?)	Hackman 1948
Pellenes spp.	23t	1sm	2t	Maddison 1982

Table 1.2 Spider species carrying non-telocentric chromosomes. m=metacentric, t=telocentric, sm=sub-metacentric.

SPECIES	AUTOSOMES	X's	REFERENCE
Micrommata viridissima	32 (?)	3	Hackman 1948
Thelticopis severa	40	3	Suzuki 1952
Heteropoda venatoria	38	3	Suzuki & Okada 1950
H. leprosa	38	3	Datta & Chatterjee 1983
H. sikkimensis	38	4	Datta & Chatterjee 1983
H. forcipata	38	3	Suzuki 1952
H. sexpunctata	20	1	Bole-Gowda 1952
Spariolensis tigris	38	3	Bole-Gowda 1952
Sparassus sp.	40	2	Datta & Chatterjee 1983
Delena cancerides	40	3	McIntosh 1949
Olios sp.	40	3	McIntosh 1949
Olios lamarcki	40	2	Bole-Gowda 1952

Table 1.3 Autosome and sex chromosome number of sparassid species.

The count for *Micrommata viridissima* is uncertain.

SPECIES	P	H	LIFESTYLE	LOCI ASSAYED	REF
Nesticus eremita	34.7	0.106	caves & surface	20	Cesaroni et al 1981
N. menozzi	22.2	0.090	troglobitic	20	"
N. sbordoni	31.6	0.109	"	20	"
Meta menardi	12.1	0.025	"	15	Nevo et al 1984
M. segmentata	71.4	-	surface	7	Pennington 1979
Araneus ventricosus	27.5	0.094	surface	30	Nevo et al 1978
Acharanea wau	3.4	-	social, surface	29	Lubin and Crozier 1985
Anelosimus eximius	13.7	0.060	social	51	Smith 1986

Table 1.4 Percentage of loci polymorphic (P) and average heterozygosity per locus per individual (H) for eight spider species.

	2n( )	Ring or chain Length	ref
Cyrsylus volkameriae (flea-beetle)	30	C IV	Virkki 1967
Nosopsyllus fasciatus (flea)	20-27	C IV	Bayreuther 1969
Austroagalloides sp. (leaf hopper)	18	C IV	Whitten 1968
Pellenes sp. (jumping spider)	26,28	C III*	Maddison 1982
Isoptera, 21sp. (termites)	32-63	R IV	Vincke & Tilquin 1978

Table 1.5 Species known to carry simple sex-linked translocation heterozygosity which results in the formation of small chains or rings.

C = chain, R = Ring. * In *Pellenes* an extra X chromosone is associated with the CIII, but this is a non-homologous association, and no chiasma is formed.

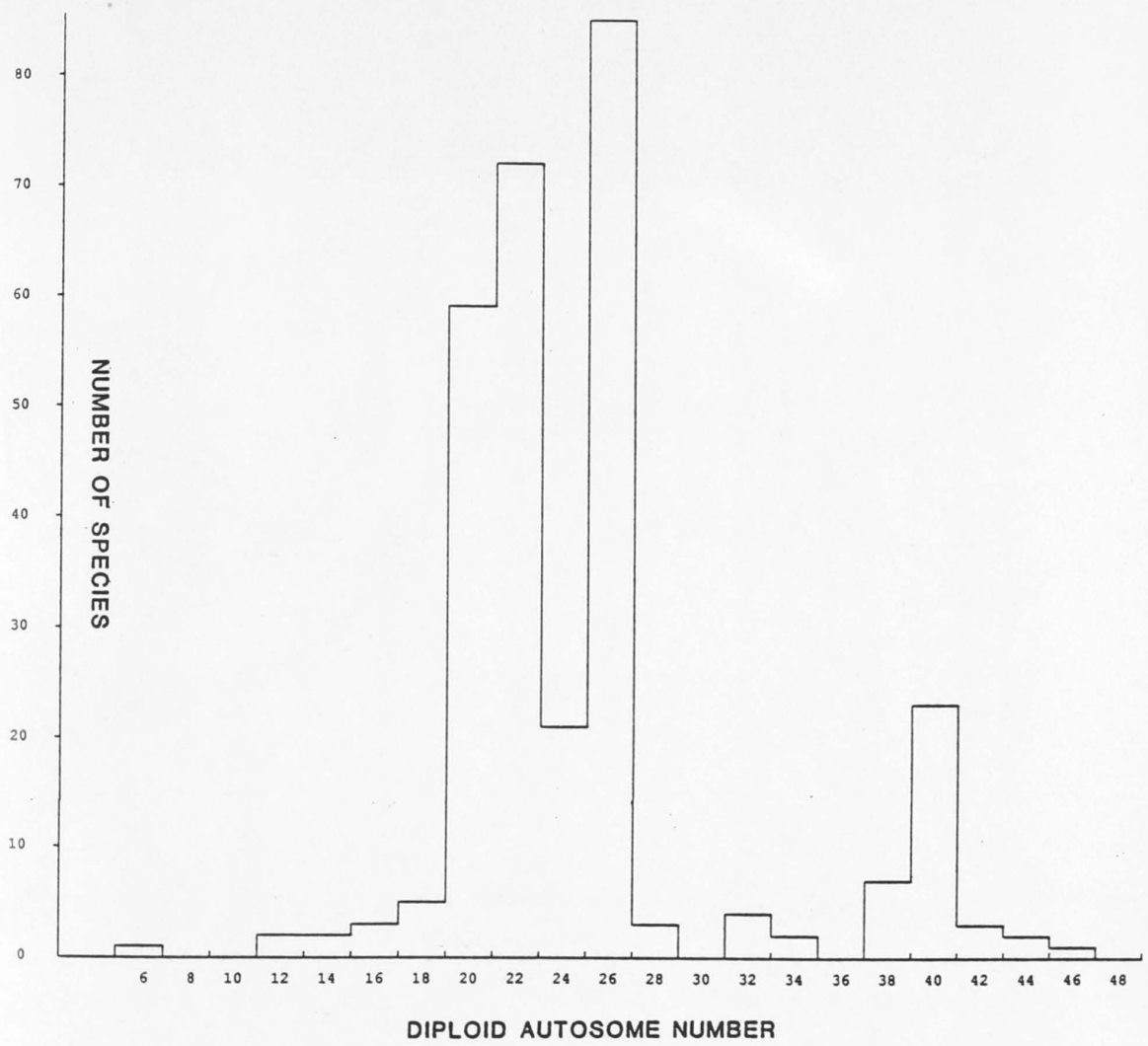


Figure 1.1 Graph showing the frequency of diploid autosome numbers in 295 species of spider that have been karyotyped. The liphistiid spider *Heptathela kimurai* (2n=92) is not included. Chromosome numbers were obtained from the following references - Bole-Gowda (1950), Bole-Gowda (1952), Suzuki (1950), Bole-Gowda (1958), Suzuki (1951), Brum-Zorilla and Postiglioni (1980), Suzuki (1952), Datta and Chatterjee (1983), Suzuki (1954), Hackman (1948), Suzuki & Okada (1950), Hard (1936), Wallace (1909), Kageyama *et al.* (1978), Wise (1983), Maddison (1982), McIntosh (1949), Mittal(1961), Mittal (1982), Montgomery (1905), Painter (1914), Patau (1948), Revell (1947), Sharma (1961), Sharma and Gupta (1956), Sharma and Singh (1957), Sharma *et al.* (1958), Sharma *et al.* (1959).

Figure 1.2 Chromosomal translocations can lead to higher order gene linkage beyond the limits of a single chromosome. In I, a single chromosomal fusion has occurred, and meiotic pairing and crossing over results in the formation of a trivalent. If the gametes produced are to be balanced, and hence viable, they must possess only one copy of each homologous chromosome arm. Thus segregation at division I of meiosis must be alternate as shown, and only two kinds of gametes will result - one carrying the A B fusion product, and the other with the two unfused A and B chromosomes. Consequently, these two unfused chromosomes are effectively linked, since they will always occur together in the gametes.

A reciprocal translocation or interchange as in II will lead to the formation of a ring-shaped quadrivalent at meiosis. In this example the ring has been opened out for convenience, but a chiasma between the two A arms would normally be expected. Again, viable gametes can only result if alternate segregation occurs, as shown. Thus chromosomes AB and CD are linked, as are BC and DA.

In both of these examples, the genetic material on these chromosomes will be linked to a greater or lesser degree depending on the number and position of chiasmata. If there is only a single terminal chiasma on each chromosome arm as shown here, gene linkage will be complete.

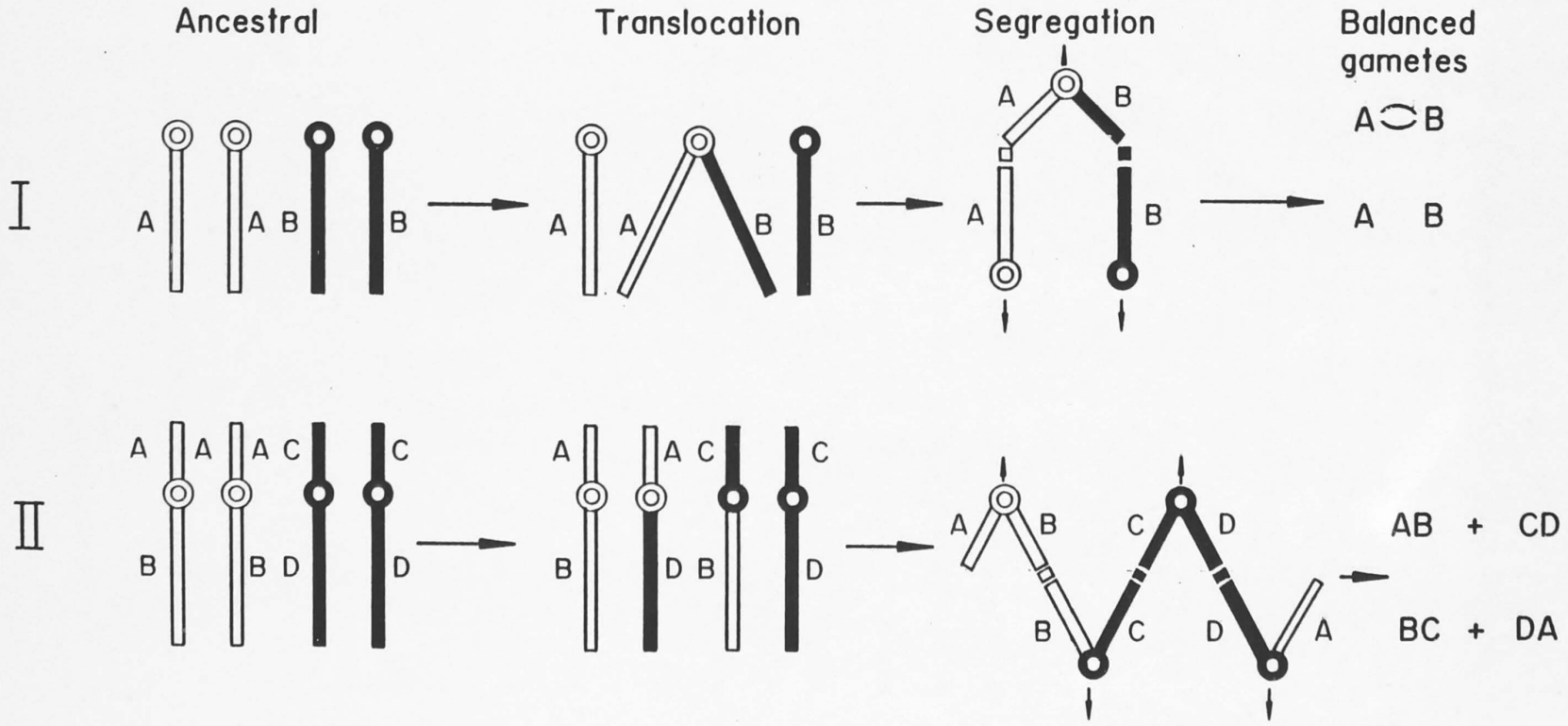
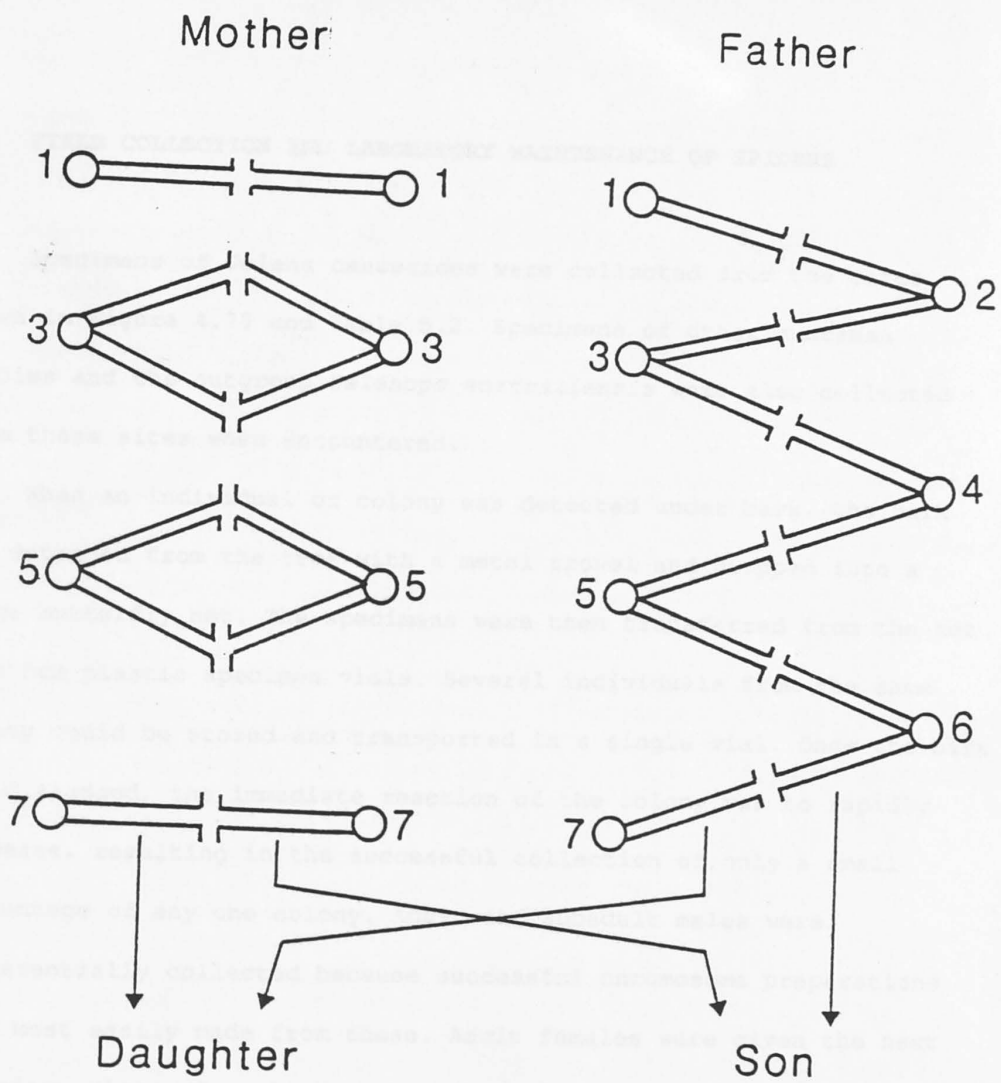

Ancestral
Translocation
Segregation
Balanced gametes
I
A A B B
A A B B
A B
A B
A B
II
A A A C C
B B D D
A A C C
B D B D
A B C D
B C D A
AB + CD
BC + DA

Figure 1.3 Schematic representation of a sex-linked translocation chain involving seven chromosomes. The chromosomes involved constitute two groups, analogous to sex chromosomes. The group consisting of chromosomes 1,3,5 and 7 behaves as an X chromosome, and so is homozygous in the female. Hence all eggs possess these chromosomes. The male is heterozygous for the two groups (1,3,5,7 vs 2,4,6), and so meiotic pairing results in a translocation chain. Chromosomes 1,3,5 and 7 are incorporated into female-determining sperm, while 2,4 and 6 behave as Y-chromosomes, and migrate together to form male-determining sperm. Thus the sex determining mechanism maintains the translocations in the homozygous state in the females and the heterozygous state in males.


Mother
Father
1
1
3
3
5
5
1
2
3
4
5
6
7
7
7
Daughter
Son

CHAPTER 2

MATERIALS AND METHODS

2.1 FIELD COLLECTION AND LABORATORY MAINTENANCE OF SPIDERS

Specimens of *Delena cancerides* were collected from the sites shown in Figure 4.78 and Table 5.2. Specimens of other huntsman species and the outgroup *Selenops australiensis* were also collected from these sites when encountered.

When an individual or colony was detected under bark, the bark was detached from the tree with a metal trowel and dropped into a large butterfly net. The specimens were then transferred from the net into 5cm plastic specimen vials. Several individuals from the same colony could be stored and transported in a single vial. Once the bark was dislodged, the immediate reaction of the colony was to rapidly disperse, resulting in the successful collection of only a small percentage of any one colony. Adult and subadult males were preferentially collected because successful chromosome preparations were most easily made from these. Adult females were given the next priority, since, for electrophoretic analysis, their sex could be determined without dissection.

Many specimens were also supplied by other collectors, generally from the walls and curtains of private homes. These were almost exclusively *Olios* and *Isopoda*.

In addition to the huntsman species, representatives of five other families were collected. An egg sac from an unidentified species of the miturgid genus *Uliodon* and a male from an unidentified species of the drassid *Rebilus* were collected from under rocks in the local area and two males of *Geolycosa godeffroyi* were collected from burrows

locally. Egg sacs from the social thomisid *Diaea socialis* from southern WA were supplied by Dr B.Y. Main as part of an unrelated project, and three male specimens of *Selenops australiensis* were collected from under bark; one each from Raymond Terrace and Grafton (NSW) and one by Dr Max King from the Darwin area.

Specimens were maintained in plastic vials with a small piece of damp absorbent cotton wool to maintain humidity and avoid desiccation which is a major mortality factor. Because the sexes are morphologically indistinguishable until after the penultimate moult, immature specimens were maintained on a diet of young grasshoppers and *Galleria* larvae until they reached this stage. In all age groups, a mortality rate of about 20% occurred during the first week after collection, and again during moulting.

Once the sex of a specimen could be determined, females were frozen in liquid N_2 until electrophoretic analyses could be carried out. Testes were removed from both adult and subaldult males for cytological study, and the body was then frozen for future electrophoretic studies.

Egg sacs of *D. cancerides* could not be maintained in the laboratory because of desiccation and fungal infection. The reason for this is not apparent, however this is a feature peculiar to many social spider species (Vollrath, pers. comm.).

For the other species, the egg sacs were kept in vials with a piece of damp cotton wool and periodically opened to monitor their stage of development prior to karyotyping.

2.2 CYTOLOGY

2.2.1 Slide preparation.

Testes from adult and subadult males were used to study male meiosis and spermatogonial mitosis. Mitotic cells from males and females were obtained from embryos when the legs became visible through the egg membrane but before any cuticle was laid down. This stage was usually reached 2 weeks after laying, but varied with incubation temperature - a higher ambient temperature resulting in more rapid development.

Testes were dissected into insect saline (Table 2.3) at room temperature and subjected to hypotonic treatment by the addition of an equal volume of double distilled water, and left to stand for 5-10 min. At this point the insect saline/distilled water mixture was replaced with a methanol:acetic acid (3:1) fixative which was repeatedly changed at 15-30 minute intervals for 3-5 h. This constant changing of fixative was found to be absolutely necessary for satisfactory fixation of dividing cells.

The fixed tissue was placed on a clean microscope slide in three drops of 60% acetic acid and macerated with the flat end of a 3mm diameter brass rod. Slides were dried on a hotplate at 37^{o}C with occasional tilting to spread the tissue. At this point the air-dried slides could be stored indefinitely before staining. Horizontal staining was carried out with 5% Giemsa (Medochrome, Melbourne) for 3 min.

The same procedure was used for embryonic tissue except that, in this case, the embryo (dissected from the yolk and egg membrane) was placed in 0.002% colchicine in insect saline for 30 min prior to hypotonic treatment.

2.2.2. C-banding.

Embryonic and testicular preparations were C-banded using the following technique:

1 - 21 day old slides were placed in a coplin jar containing saturated (5%) barium hydoxide for 3-5 min. They were rinsed in two changes of distilled water and one of 0.2M HCl, and then placed in coplin jars of 2 x SSC (Table 2.3) at 60°c for 25-35min. After two washes in distilled water, the slides were immediately stained for 7 min with 5% Giemsa stain in phosphate buffer (Table 2.3). Slides which had previously been gross stained and scanned for dividing cells could subsequently be C-banded after removing all traces of immersion oil by washing the slides in xylene.

2.2.3 Other banding techniques.

G-banding, N-banding and NOR staining were also attempted using standard techniques but with no success. N. Contreras and M. Arnold attempted *in situ* hybridisation of *D. cancerides* and *Isopoda villosa* chromosomes using cloned *Drosophila* 18S and 26S rDNA, and 26S rDNA from the grasshopper *Caledia captiva*. No hybridisation of the probes to the chromosomes was evident, presumably because of insufficient sequence homology between the spider and insect species.

2.3 SYNAPTONEMAL COMPLEXES

Synaptonemal complexes were prepared for light microscopic analysis using testes from adult and subadult males of *D. cancerides* and *Isopoda villosa*. The techniques for slide and tissue preparation, fixation, and spreading were modified from those of Gillies and Peters (pers. comm.).

2.3.1. Slide preparation.

The best results were obtained after the slides were first made hydrophilic. To achieve this, two techniques were used:

(a) Plastic coating. A 10g plastic petri dish was dissolved in 500ml of chloroform. Washed slides were dipped in this solution and drained vertically.

(b) Gelatin coating. Washed slides were dipped in a solution of 0.1% gelatin and 0.01% chrome alum, then allowed to dry vertically.

Plastic coated slides produced better spreading, but the plastic tended to come off the slides during washing, and disintegrated when the slides were coverslipped.

2.3.2. Tissue preparation and fixation.

Testes were dissected into 0.5ml of insect saline. The tissue was macerated using a 3mm thick brass rod to produce a uniform cell suspension. A single drop of the suspension was placed in the middle of a plastic or gelatin coated slide with two drops of 0.03% "Trix" lemon dishwashing liquid. After 3-5min, 6 drops of paraformaldehyde fixative (Goodpasture and Bloom 1975) were added, and the slide placed on a hotplate to dry. After drying, the slides could be stored indefinitely before staining.

2.3.3. Staining.

Before staining for synaptonemal complexes, slides were pretreated in double distilled water for 1 min to remove excess fixative and then dipped in 0.4% Photoflo (Kodak) before draining vertically. Three staining techniques were used:

(a) Silver staining. One drop of 50% silver nitrate was placed on each slide, and spread by applying a 22mm x 22mm coverslip. The slides were left overnight in a sealed plastic lunchbox on a damp tissue to maintain humidity. After washing in distilled water to remove the coverslip and excess silver nitrate, the slides were drained and mounted with Euparal. DPX was not used because it dissolved the plastic coating. This technique tended to result in patchy, uneven staining and excessive background, and was consequently abandoned.

(b) Ammoniacal silver staining (Goodpasture and Bloom 1975). This technique resulted in a minimum of background and maximum contrast, but was unreliable. The tissue stained unevenly, and only small areas showed a satisfactory intensity of staining. Hence this technique was only used when sufficient tissue was available from a single individual to produce a large number of slides.

(c) Giemsa staining. Slides were stained for 20min with 10% Giemsa stain in phosphate buffer. Spreads stained using this technique showed less contrast than ammoniacal silver staining, but since it was completely reliable it was generally used.

2.4 PROTEIN ELECTROPHORESIS

Electophoresis was carried out using cellulose acetate gels ("Cellogel", Chemetron,Milan).

A mixture of leg muscle and digestive gland was used initially, but as the presence of digestive gland tissue caused smearing of bands and inhibition of aldolase, only leg muscle was subsequently used.

30 protein systems were assayed for activity and clarity of bands using TC100 and TEM50 buffers (Tables 2.1, 2.3). Systems yielding discernible bands were then tested on TC80, TEM15, and TEB15 running buffers (Table 2.3) for maximum clarity. Final running buffers and conditions are presented in Table 2.2 .

2.4.1. Sample preparation.

Approximately 0.4 g of tissue (whole legs) was ground in 0.75ml of grinding buffer in glass grinding tubes, and spun for 3 min in an Eppendorf centrifuge. The supernatant was removed and stored in liquid N_2 until use.

2.4.2. Running.

Gels were soaked in the appropriate running buffer for 15 - 120 min before use. 1 - 2µl of each sample was loaded cathodally with a mapping pen onto the blotted, mounted gel. Application was repeated every 10 - 15 seconds for weaker systems. Up to 25 samples could be loaded onto a single 10cm gel.

2.4.3 Staining.

Table 2.2 gives references for the staining recipes used. Gels were placed face down on 1ml of staining solution for 2 - 5 min, blotted, and incubated in a humidified plastic container at 37°C.

Development was halted in 3% formaldehyde. Formazin-stained gels were stored in sealed plastic bags containing a few drops of 7% acetic acid. Other gels were stored in 7% glycerol.

2.4.4 Scoring.

Gels were scored as soon as possible after formaldehyde fixation, and in all cases within 24hr, since stored gels sometimes faded. After all samples had been run, relative migration rates were ascertained using comparison gels, and the alleles scored alphabetically in order of migration rate, from the fastest to the slowest.

2.4.5 Data analysis.

Electrophoretic data was analysed using the BIOSYS package (Swofford and Selander 1981) on the RSBS Vax 660 computer. The statistical techniques used will be described in Chapter 5.

PROTEIN	ABBREVIATION	E.C. NO.	STAINING
Alcohol dehydrogenase	ADH	1.1.1.1	4
Glycerol phosphate dehydrogenase	GPD	1.1.1.8	3 *
Lactate dehydrogenase	LDH	1.1.1.27	4 *
Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30	1
Malate dehydrogenase	MDH	1.1.1.37	1 *
Malic enzyme	ME	1.1.1.40	1 *
Isocitrate dehydrogenase	IDH	1.1.1.42	1 *
6-phosphogluconate dehydrogenase	PGD	1.1.1.44	3 *
Glucose-6-phosphate dehydrogenase	Gd	1.1.1.49	3 *
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	1 *
Glutamate dehydrogenase	GDH	1.4.1.3	3
Aspartate amino transferase	AAT	2.6.1.1	2 *
Glutamate-pyruvate transaminase	GPT	2.6.1.2	1
Hexokinase	HK	2.7.1.1	1 *
Phosphoglycerate kinase	PGK	2.7.2.3	1
Adenylate kinase	AK	2.7.4.3	3(A) *
Phosphoglucomutase	PGM	2.7.5.1	1 *
Esterase	EST	3.1.1.1	3(A,B) *
Acid phosphatase	ACP	3.1.3.2	1
Peptidase Leu-Gly-Gly	PEP	3.4.11	3(A)
Peptidase Leu-Ala	PEP	3.4.11	3(B)
Guanine deaminase	GDA	3.5.4.3	3
Aldolase	ALD	4.1.2.14	1 *
Fumarase	FUM	4.2.1.2	1 *
Aconitase	ACON	4.2.1.3	3
Triose phosphate isomerase	TPI	5.3.1.1	3(B)
Mannose phosphate isomerase	MPI	5.3.1.8	3 *
Glucose phosphate isomerase	GPI	5.3.1.9	1 *
Phenol oxidase	PO	-	
General protein	GP	-	*

Table 2.1 Enzyme systems tested electrophoretically in *Delena*. * denotes detectable activity. 1=Moran *et al.* 1980,2=Shaw & Prasad 1970,3=Harris & Hopkinson 1976,4=Richardson *et al.* 1986.

ENZYME SYSTEM	RUNNING BUFFER	NO. OF LOCI	RUNNING TIME (AT 7mA)
GPD	TEM50	1	1.5hr
LDH	TC80	1	2hr
MDH	TEM50	2	1.5hr
IDH	TC80	2	2hr
PGD	TEM50	1	1.5hr
Gd	TEM50	1	1.5hr
GAPDH	TEM50	1	1.5hr
MPI	TEM50	1	2hr
AAT	TC80	2	1.5hr
HK	TEM50	1	1.5hr
AK	TEM50	1	1.5hr
PGM	TEB15	1	1.5hr
ALD	TEM50	1	1.5hr
FUM	TC80	1	2hr
GPI	TEB15	1	1.5hr
GP	TEM50,TEM15	3	1.5hr

Table 2.2 List of enzymes examined, number of loci, and running conditions.

Table 2.3 Solutions and buffers.

Insect saline:
7.0g NaCl, 0.2g $CaCl_2$ per litre of solution

2 X SSC:
17.52g NaCl, 8.80g Na citrate per litre of solution
pH to 7.2 with NaOH or HCl

Phosphate buffer:
1g KH_2PO_4, 2.0g $Na_2HPO_4.12H_20$ per litre of solution
pH to 6.8 with NaOH or HCl

Grinding buffer:
1.21g Tris, 0.04g EDTA, 0.005g NADP, 50 l β-mercaptoethanol per 100ml H_2O
pH to 7.0 before adding β-me

TEM15:
1.82g Tris, 0.2g EDTA, 0.2g MgCl per litre of solution
pH to 7.8 with maleic acid

TEM50:
6.05g Tris, 0.37g EDTA, 0.2g MgCl per litre of solution
pH to 7.8 with maleic acid

TEB15:
1.82g Tris, 1.86g EDTA per litre of solution
pH to 7.8 with boric acid

TC80:
19.36g Tris per litre of solution
pH to 8.2 with citric acid

TC100:
24.20g Tris per litre of solution
pH to 8.2 with citric acid

CHAPTER 3

GENERAL OBSERVATIONS AND SOCIAL BEHAVIOUR OF THE SPARASSIDAE.

The main aims of this work centre on the analysis of electrophoretic variation and chromosomal morphology and behaviour in the sparassid spiders. A number of general observations were made on the life histories and behaviour of the species collected, both in the field and during their maintenance in the laboratory, which are relevant to the genetics of this group and which the reader will need to be aware of before being presented with the chromosomal and electrophoretic data.

3.1 GENERAL OBSERVATIONS.

Figures 3.1 to 3.8 illustrate representative specimens of the genera collected.

Table 3.1 summarises the observations made on the behaviour and habitat of the species examined. Table 3.2 gives details of the collection localities for species of *Pediana, Olios, Isopoda* and *Heteropoda*, while Figure 4.78 and Table 5.2 show the collection sites for *Delena cancerides*.

3.1.1 Habitat.

Of the five genera, *Delena* and *Isopoda* are the most similar in terms of their abundance, habitat preference, size and body form. Indeed members of these two genera may compete for resources. Although no active monitoring of wild populations was carried out, it was noted that, generally, when *D. cancerides* was present on eucalypt species,

Isopoda species were absent or rare. In areas where one or more species of *Isopoda* were abundant, *D. cancerides* was usually found only under the bark of dead *Callitris, Acacia* (Fig. 3.9), *Banksia* and *Casuarina* species, rather than on the eucalypt species which are preferred by *Isopoda*. When *Callitris, Acacia, Banksia* and *Casuarina* species die, the trunk contracts, leaving a narrow, uniform gap between the bark and the trunk, around the full circumference of the tree. This provides a large area under cover, which is too narrow for large *Isopoda* species to enter, owing to their more raised carapace.

3.1.2 Parasitism.

All of the genera studied (and indeed all of the species collected) possess small white or orange body mites (Table 3.1). These mites are kleptoparasites, leaving the spider's body to congregate on its prey and feed. It is not clear whether they also directly parasitise the spider. These mites appeared to be less common on members of *D. cancerides* colonies than on solitary individuals and solitary species, but this observation was not quantified and requires further investigation.

Two mantispid larvae were discovered in a *D. cancerides* egg sac and two in an egg sac of *Isopoda vaster*. McKeown and Mincham (1948) and Austin (1985) also reported egg parasitism by *Mantispa vittata* and *Austromantispa* sp. in unidentified *Isopoda* species. Hickman (1970) recorded a dipteran egg parasite of *D. cancerides, Gaurax delenae*.

A 10 cm mermithid worm (Nematoda) was collected from the abdomen of a juvenile male *D. cancerides*. The worm had eaten most of the digestive gland, fat and testes, but had left the gut and heart intact.

A larva of the pompilid wasp *Platyderes collaris* was found on the body of an active adult female *Pediana regina* (Fig. 3.6). Within 3

days, the whole body of the spider had been eaten, leaving only cuticle. The wasp pupated and was kept alive until it emerged, for identification purposes.

3.1.3 Egg sacs.

The egg sacs of the five genera are described in Table 3.1, and a *Pediana* egg sac is pictured in Fig. 3.5.

Except for *D. cancerides*, all of the genera construct very similar egg sacs, encased in a parchment-like silk membrane and loosely attached to the substrate. In the laboratory, eggs which were removed from the sac died from desiccation within 48 hours unless high humidity levels were maintained. Austin (1985) has suggested that the egg sac wall may be an adaptation for protection against egg parasites.

In *D. cancerides*, the eggs are contained in fine, loosely woven silk, and securely anchored flat to the trunk. This appears to afford less protection to the eggs than the denser egg sac wall of the other genera, but it may reflect a lowered risk of desiccation and parasitism both because of the more confined spaces often inhabited by *D. cancerides*, and because of its social behaviour.

In *Isopoda immanis* (Fig. 3.1), there is evidence that the female opens the egg sac for the young to emerge (Coleman 1941). Winsor (1972) reported on a similar phenomenon, however in this instance, the egg sac which she claimed to be from *D. cancerides* was clearly misidentified since the egg sac she illustrates is quite unlike that of *D. cancerides*.

3.1.4 Social behaviour.

(i) Intraspecific interactions in *Pediana* and *Isopoda*. In these two genera, some degree of intraspecific tolerance is evident. Females

remain in close proximity to their egg sac, and tolerate the presence of and physical contact with the hatchlings for two to three weeks. In *Pediana regina*, the hatchlings congregate on the body of the mother.

Male/female pairs are occasionally encountered in close proximity or even touching. Coleman (1941) was able to maintain a male and a female of *Isopoda immanis* in the same container for over five months before the female killed and ate the male. This is unusual, however, and generally when two conspecifics are housed together, one is killed and devoured.

(ii) Colonial behaviour in *D.cancerides*. In contrast to the intolerance of conspecifics in species of the other genera, *D. cancerides* (Fig. 3.7, 3.8) is social. Although solitary individuals (usually adult females) are often encountered, colonies also exist, which generally consist of a single female and a number of size classes of juveniles, possibly her offspring from successive egg layings, all in close contact. Colonies of up to 300 juveniles, six adult females and three adult males are also encountered. In these very large colonies all instars may be present, ranging from hatchlings to adults. This is rare in social species (Buskirk 1981). Furthermore, when multiple adult females are present, they may include old individuals (as evidenced by size, greater melanisation and hairiness) as well as young adults barely larger than juveniles. These may represent mothers and daughters, and because more than one egg sac may be present in these colonies, they are presumably all reproductives; this could be verified by examination of the sperm receptacles. Thus *D. cancerides* colonies appear capable of supporting overlapping generations. This phenomenon, also rare in social spiders (Buskirk 1981), is one of the three criteria which have been used to define eusocial behaviour as observed in the Hymenoptera and Isoptera (Michener 1974). There is no evidence, however, that *D. cancerides*

fulfills Michener's other two criteria for eusocial behaviour, namely specialised adult brood care and the presence of morphological castes.

The age structure of colonies is pyramidal, with the youngest cohorts being the most numerous and the older age classes rarer. This is most probably due to attrition with age from mortality or dispersal.

When the bark surrounding a *D. cancerides* colony is removed, the colony scatters in all directions. In several instances when a colony site was revisited days, weeks or months after the collection of a colony, individuals or groups of two or three spiders only were found. Hence it appears that colonies which have been disturbed do not reaggregate. This precludes any field monitoring of colonies.

(iii) Home maintenance. In *Isopoda* and *Pediana*, females with egg sacs seal themselves into a web cell under bark, and aggressively protect the egg sac and hatchlings until they disperse. In *Pediana regina*, small pieces of bark and debris are sometimes attached to the outer side of the cell for camouflage (Fig. 3.6). Other than at this time, no "nest" is built. Thus, when a sheltering piece of bark falls from a tree, another suitable site must be found. In *D. cancerides* colonies, on the other hand, the bark is firmly attached to the tree with very tough golden-brown silk. The same distinctive silk is used to patch splits in the bark, perhaps to keep out rainwater or to prevent the bark from splitting further (Fig. 3.10). This means that the bark remains attached to the tree for much longer than it would naturally, and consequently the potential life of the colony may be considerably increased. Because this webbing is present even in very young colonies, it is presumably produced by the female(s). Although no *D. cancerides* specimens have been reared from eggs to adulthood in the laboratory, given the growth rate of specimens that have been maintained for three or four months, it is clear that it takes more

than one year, and possibly two or three, for this species to reach maturity. Hence colonies with two generations of reproductives may be at least two years old. If daughters remain with the colony or recruitment of outsiders occurs, colonies could theoretically last indefinitely, or until the bark falls off or some other natural disaster occurs.

3.2 INTRASPECIFIC TOLERANCE, PREY CAPTURE AND FEEDING IN *D. CANCERIDES*

In other species of social spiders, all conspecifics are generally tolerated (Darchen and Delage-Darchen 1986). In *D. cancerides*, however, this is not the case. While individuals from the same colony could be collected and maintained in the same container, it was very quickly discovered that members of different colonies housed in the same container often killed and ate each other.

Since preference was given to electrophoretic and chromosomal analyses, spiders were generally killed and frozen shortly after collection. However, when large samples were available a limited investigation of tolerance, prey capture and colony feeding behaviour was possible. Although these experiments were necessarily simple and often incomplete, some conclusions can be drawn regarding these behaviours.

3.2.1 Conspecific tolerance.

Experiments and observations on conspecific tolerance are described below.

(i) In an attempt to breed *D. cancerides* one adult male/female pair, collected separately from Kioloa (NSW), was placed in each of

six wood and screen wire cages (15" x 15" x 24") with several large pieces of bark, a petri dish of damp cotton wool and a number of *Galleria* larvae for food. These cages were checked after three days, and in every case the male had been killed and eaten by the female.

(ii) Two specimens - a subadult male from Bugaboi (Vic) and a small juvenile from Bega (NSW) - were placed in a jar together. The next morning the small juvenile had been eaten.

(iii) An adult female from Tomakin (NSW) and a juvenile from Merry Beach (NSW) were placed in a jar together. The next morning the juvenile had been eaten, and the female had lost four legs.

(iv) Two jars containing three and four juveniles collected from the same colony from Kioloa (NSW) were kept separate for three months. After this period, the jars were combined. The spiders aggregated, all in physical contact with at least one other individual and no aggressive behaviour was observed. After a week, all were still alive.

(v) A juvenile collected from a colony from Kioloa (NSW) was placed in a jar with three juveniles collected from another colony 20 metres away. It was immediately killed.

(vi) A colony collected from Greenways (SA) of approximately 50 individuals consisting of four distinguishable cohorts of juveniles and an adult female was maintained in a large glass jar in the laboratory for two months. At this point, two juveniles from Glenthompson (Vic), slightly smaller than the largest cohort, were placed in the jar. These remained together but separate from the rest of the colony for about two weeks. After this time they joined the main aggregation and their presence was tolerated. Shortly afterwards, the adult female died (for no apparent reason) and was replaced with an adult female from Glenthompson (Vic). Over the next five days, the female killed all of the colony members except for three, two of which had previously become adult males, and one subadult male. These four

spiders remained together for almost six weeks, when the female laid a small egg sac, and the males were removed. Two weeks later eleven hatchlings emerged, but died within a week.

From these observations it is clear that recognition in *D. cancerides* is colony specific, and even animals from nearby colonies can be recognised as foreign. Since the juvenile in (v) was collected from the nearest tree to the colony into which it was introduced, it may well have been closely related. Thus the fact that it was not tolerated suggests that recognition is based on colony "smell" rather than any specific kin recognition mechanism.

The two juveniles in (vi) from Glenthompson were clearly unrelated to the Greenways colony due to the distance between the two sites. They were probably adopted into the colony because they escaped detection for long enough to acquire the colony "smell". The fact that the female from Glenthompson left three males is probably due to mate tolerance, also seen in other huntsman species. This may be pheromonal or related to the behaviour of the males.

3.2.2 Colony protection, prey capture and feeding.

D. cancerides is a very nonaggressive species, and when a colony is disturbed, no concerted effort is made to defend the nest. Adult females, particularly those with eggs or hatchlings, may attempt to bite, however.

When small juveniles were given prey, often more than one attacked, although whether this represented a concerted effort or independent attacks could not be discerned.

In the colony described in (vi) above, prey was shared, but grudgingly. If two or three members attacked a *Galleria* larva, a tug-of-war ensued. After a few seconds, however, all of the members

involved settled down to feed on the same item and no aggressive behaviour was observed.

3.2.3 Foraging.

Because *Isopoda* and *Pediana* are territorial, only a small number of individuals are usually present on any given tree. Hence it is surprising that a single small tree can also support a whole *D. cancerides* colony. Under these circumstances, long-range foraging would be expected to be necessary in order to feed the colony. This was never observed during daylight hours, however, and no spiders were seen coming or going from three colonies from Bugaboi (Vic) which were monitored by torchlight from 10pm to 1am in early May, 1984.

3.2.4 Dispersal.

While it appears that some females may remain with their colony after maturity, the fact that solitary individuals are often found implies that dispersal does occur. Furthermore, since acceptance of foreign males by a female has been observed in the laboratory, it is possible that this also occurs in nature, and hence there may be some degree of outbreeding.

3.2.5 Sex ratio.

In section 1.5 it was mentioned that the sex ratio in social spiders, where it has been analysed, is always skewed in favour of females. It was intended to analyse this by karyotyping embryos, since in this way, any effects of sex-specific mortality can be removed. This was not possible, however, because no egg sacs from *D. cancerides* were obtained at the appropriate stage of development and they could not be maintained in the laboratory. Instead, hatchlings from four colonies were dissected and their sex determined. Unfortunately, at

this stage the spiders are very small, and the gonads are often too underdeveloped to distinguish testes from ovaries. Consequently the number of individuals successfully sexed was low. These data are given below:

LOCALITY	MALES	FEMALES	TOTAL
Tamworth NSW	14	15	29
Kuringai NSW	4	1	5
Greenways SA	6	6	12

Clearly these data do not deviate significantly from a sex ratio of 1:1.

3.3 DISTRIBUTION AND ABUNDANCE OF *D. CANCERIDES*

As has already been mentioned, *D. cancerides* appears to be present in all suitable areas across Australia and its offshore islands. Population densities varied markedly, however. Almost every suitable tree sampled in south eastern Victoria (Fig. 4.78) harboured at least one colony, but in the Gosford area, colonies and individuals were extremely rare. In the coastal forests north of Cann Valley (Vic) which had suffered bushfires two years before, no specimens were found, even after intensive searching, because there were no trees with suitable bark exfoliations.

3.4 DISCUSSION

The behaviour of *D. cancerides* is unique, and in some respects it may be more highly evolved than other social spiders. These include the tolerance and recognition of other colony members even after three months of separation, the recognition of and aggression towards non-member conspecifics, and the overlap of generations. This behaviour is most probably derived in part through neoteny since mature individuals and large juveniles are maintaining, apparently indefinitely, the behavioural patterns peculiar to hatchlings in other huntsman species.

The advantages for *D. cancerides* of living colonially may include the capture of larger prey (and hence a wider prey range) and the increased probability of finding a mate. Also, because dispersal of offspring is not obligatory as it is in other huntsman species, colony members have the freedom to remain with the colony until optimal conditions for dispersal occur. Furthermore, dispersal at a larger size (and age) may increase their chances of survival.

A potential disadvantage of colonialism is that it may be necessary for colony members to forage further afield than solitary species, due to the exhaustion of local resources. There is no evidence that this occurs, however.

	HABITAT	PARASITES	EGG SACS	TOLERANCE	COOPERATIVE PREY CAPTURE & FEEDING
Delena	Under bark on dead *Callitris, Acacia,* and *Banksia.* Less often on *Eucalyptus.* Occasionally under rocks. Rarely in houses.	Body mites, mermithid worms internally, mantispid larvae in egg cases.	Lens shaped, fine silk. Strongly attached flat on tree trunk. Guarded by female.	Tolerate all immediate kin. Conspecifics from other trees generally not tolerated.	✔
Pediana	Under bark or in crevices, generally on *Eucalyptus.*	Body mites, pompilid wasps - *Platydeces collaris.*	Rounded, lens-shaped. Parchment-like silk. Loosely attached to trunk by silk lines. Guarded by female.	Mating pairs found together. Mother guards hatchlings. Otherwise, cannot be housed together.	×
Isopoda	Under any loose bark, especially on *Eucalyptus.* Common in houses.	Body mites, mantispid larvae in egg sacs.	Flat, lens-shaped. Parchment-like silk. Loosely attached to trunk by silk lines. Guarded by female.	" "	×
Heteropoda	Under loose bark, in foliage.	Body mites.	Lens-shaped, flat to rounded. Attached to bark or foliage by silk lines. Guarded by female.	" "	×
Olios	Under bark, in foliage. Common in houses.	Body mites.	Flat, lens-shaped to spherical. Attached to underside of bark or foliage by silk lines. Guarded by female.	" "	×

Table 3.1 Ecological and behavioural observations on the five genera of huntsman spiders collected.

Isopoda immanis	Brou Lakes (NSW), Kilcoy (Qld), Atherton tableland (Qld), Cudal (NSW), Brou Lakes (NSW), Yellow Pinch Ck (NSW), Armidale (NSW), Grafton (NSW), Tenterfield (NSW).
Isopoda insignis	Broken Hill (NSW), Balranald (NSW), Narrabri (NSW).
I. villosa	ACT.
I. vaster	ACT, Captain's Flat (NSW), Kioloa (NSW), Merry Beach (NSW), Broulee (NSW), Bega (NSW), Quarantine Bay (NSW), Yellow Pinch Ck (NSW), Cann Valley (Vic), Lakes Entrance (Vic).
I. tepperi	ACT, Clare (SA), Adelaide (SA), Melbourne (Vic).
I. sp. 1	Balranald (NSW), Bugaboi (Vic), Melbourne (Vic).
I. sp. 2	Perth (WA).
Olios diana	ACT, Kangaroo Is. (SA), Trangie (NSW), Armidale (NSW), Numeralla (NSW), Wee Jasper (NSW), Hay Plain (NSW).
O. sp. 1	ACT.
O. sp. 2	Urapunea (NT).
Pediana regina	Nyngan (NSW), Neara Ck (Qld), Bongmuller Ck (Qld), Cherwell Ck (Qld), Gin Gin (Qld).
P. sp. 1	ACT.
Heteropoda procera	Brisbane (Qld), Sydney (NSW).
H. sp. 1	Jakiluka Billabong (Qld).

Table 3.2 Species and collection localities for the genera *Isopoda, Pediana, Olios* and *Heteropoda*.

Figure 3.1 *Isopoda immanis*, juvenile female. This is one of the largest Australian huntsman species. *I. immanis* is easily identified by the longitudinal black stripe on the dorsal surface of the abdomen. It is found throughout eastern Australia, but most commonly in eastern Queensland. Members of the genus *Isopoda* have a relatively flattened carapace, and live primarily under bark. This specimen was collected from Atherton Qld. 0.75 x life size.

Figure 3.2 *Heteropoda procera*, adult female. The genus *Heteropoda* is found mainly in northern Australia and Southeast Asia, particularly in rainforest areas. The carapace is more raised in this genus than in *Delena*, *Isopoda* and *Pediana*. Species of this genus live under bark, in foliage and in leaf-litter. Brisbane Qld. x2

Figure 3.3 *Olios diana*, adult female, dorsal view. This species has an Australia wide distribution, and is commonly found in houses. Members of this genus possess a raised carapace, and often distinctive markings on the underside of the abdomen (Fig. 3.4). They are generally found under bark or in foliage. Canberra ACT. x 1.25

Figure 3.4 *Olios diana*, adult female, ventral view. Canberra ACT. Life size.

Figure 3.5 *Pediana regina*, adult female with egg sac. Spiders of this genus are genarally smaller than those of the other genera pictured, and live exclusively on trees, either under bark or in knotholes. Nyngan NSW. Life size.

Figure 3.6 *Pediana regina*, adult female with larva of parasitic pompilid wasp (*Platyderes collaris*. Before laying eggs, females of this species build a web coccoon under bark to which small fragments of bark are attached. This individual was not paralysed when collected, but was unable to remove the larva which eventually killed it. Neara Ck Qld. x2

Figure 3.7 *Delena cancerides*, subadult male, dorsal view. In this species, the sole member of the genus (but see section 1.30), the flattening of the body and laterigrade orientation of the legs is extreme. Dorsal abdominal patterning is highly variable, ranging from a distinct fishbone outline to irregular spots, and occasionally plain olive/brown. Evandale Tasmania. x2

Figure 3.8 *Delena cancerides*, adult female, ventral view. derwent Bridge Tasmania. x1.5

Figure 3.9 Dead *Acacia* species, from which a colony of *D. cancerides* was collected. When *Acacia, Callitris, Banksia* and *Casuarina* species die, the trunk contracts, leaving a gap under the bark. At later stages, as in this photograph, the bark begins to crack, but the fragments are usually large enough to house colonies. On this tree, the large strip of bark facing had been attached to the tree using the coarse golden webbing shown in the next figure.

Figure 3.10 The coarse golden silk peculiar to *D. cancerides* is used to secure bark to trees, and is also layed across cracks either as waterproofing or to prevent further disintegration of the bark. x10

CHAPTER 4.

CYTOLOGY

Gross stained and C-banded chromosome preparations were produced for the five sparassid genera, the drassid genus *Rebilus*, the miturgid genus *Uliodon* and for the lycosid *Geolycosa godeffroyi*. Only gross stained mitotic preparations were obtained for the thomisid *Diaea socialis*.

4.1 CHROMOSOMES

4.1.1 Mitosis and meiosis in *Rebilus*, *Uliodon*, *Geolycosa godeffroyi*, *Diaea socialis* and *Selenops australiensis* : Results and discussion.

(a) Mitosis

All of these species showed typical spider karyotypes and chromosomal behaviour. The karyotypes are composed entirely of telocentric chromosomes with terminal C-band positive regions (Figs. 4.1 - 4.6). Chromosome morphology, number, size and C-band patterns are summarised below.

SPECIES	2N (male)	C-BANDS	SIZE (μ)	NUMBER ANALYSED
Uliodon sp.	24 + 2X	centro, telo	3	5
G. godeffroyi	18 + 2X	centro	2 - 5	2
Rebilus sp.	26 + 2x	centro, telo	1 - 4	1
D. socialis	22 + 2X	not banded	2 - 5	18
S. australiensis	26 + 2X,3X	centro, telo	2 - 3	3

In the *Uliodon* specimens approximately half of the chromosomes had C-band positive centromeric regions of varying intensity (Fig. 4.1), but these could not be consistently scored, since the paler bands were visible in some cells but not in others. Similarly, telomeric C-bands were also visible in some cells but not in others. In the *Rebilus* specimen centromeric C-bands were consistently visible on every chromosome in all cells (Fig. 4.3) and, in some cells, a telomeric C-band was visible on every chromosome. The centromeric regions in *G. godeffroyi* consistently C-banded but no telomeric bands were seen (Fig. 4.2). No successful C-banded preparations could be obtained from *D. socialis*.

For *Uliodon* and *Diaea*, chromosomal preparations from embryos derived from a single egg sac were used and hence meiosis was not observed in these species. The number of X-chromosomes was inferred from the fact that two different chromosome numbers were observed in the embryos - 26 and 28 in *Uliodon*, and 24 and 26 in *Diaea*. Thus these species most probably possess a sex-determining system of the $X_1X_2/X_1X_1X_2X_2$ type. An unusual feature of the *D. socialis* karyotypes was that in the 2n=26 (female) embryos, three additional microchromosomes were present (Fig. 4.5), and in the 2n=24 (male) preparations only two were seen (Fig. 4.4). If the sex determining mechanism is normal, as has been assumed here, these microchromosomes could not segregate at meiosis to produce the same numbers in the next generation. Consequently, it is most probable that these are B-chromosomes with some affinity to the X-chromosomes. It would be necessary to examine more material to conclusively demonstrate this.

(b) Meiosis

Meiotic preparations obtained from *G. godeffroyi*, *Rebilus* and *S. australiensis* are shown in Figures 4.7 - 4.10.

Of the specimens of *S. australiensis* examined, one (Darwin, NT) possessed two X-chromosomes, while the other two (Grafton and Raymond Tce, NSW) had three (Figs 4.9, 4.10). This may represent regional variation, or the individual from Darwin may have been aberrant. In all of the other species, only two X-chromosomes were present.

In all of these species normal bivalents were formed, each with a single terminal or interstitial chiasma. C-banded meiotic cells were obtained for *G. godeffroyi*, the 2X *S. australiensis* individual and *Rebilus*, and from these it is clear that in *G. godeffroyi* the majority of the terminal chiasmata are proximal, in *S. australiensis* they are mainly interstitial, while in *Rebilus* they are distal (Figs. 4.7 - 4.9).

In *Rebilus* and the 3X *S. australiensis* specimens the X-chromosomes remained closely associated and migrated as a group to one pole at first anaphase (Figs. 4.8, 4.10). In *G. godeffroyi* and the 2X *S. australiensis* individual they were usually found near each other, but not closely associated (Figs 4.7, 4.9), although they both segregated to the same pole.

4.1.2 Mitosis and meiosis in *Isopoda*, *Heteropoda*, *Olios*, and *Pediana* : Results and discussion.

(a) Mitosis.

Figures 4.11 - 4.16 show mitotic cells from species of the four genera, and these data are summarised in Table 4.1. Although the chromosomes appeared to be maximally contracted, no typical metaphase cells with the chromosomes aligned on the spindle were observed, even in preparations not treated with colchicine.

In all cases the chromosomes were telocentric and approximately 4μ in length. Slight size variation of the chromosomes was apparent within cells, but this was not sufficient to distinguish homologous pairs. In mitotic preparations obtained from embryonic tissue the chromatids were always closely associated (Figs. 4.11, 4.13), but in spermatogonial divisions they were often splayed apart, more granular in appearance, and usually only joined at the centromere (Fig. 4.12).

C-band positive material was always present on one end of each chromosome, and many of the chromosomes possessed a C-band on both ends. In C-banded meiotic anaphase II cells of *Isopoda* species (Figs. 4.17, 4.18) all of the centromeres possessed centromeric heterochromatin. Although some very large distal C-bands were present, in most cases the telomeric C-bands were smaller than those on the centromeres, and often so small that they could not be consistently scored, the number visible varying even among cells on the same slide.

At mitotic prophase the chromosomes were often non-randomly aligned across the nucleus, with most of the C-band material clustered at one side of the cell and a small amount on the opposite side (Figs 4.19, 4.20). The proportions of this material on each side of the cell were consistent with the former being the centromeres and the latter telomeres.

All of the species possessed 43 chromosomes in the male except *Heteropoda procera* which had 41. Otherwise there were no visible differences in the karyotypes. The chromosomes of *H. procera* have the same morphology, C-band distribution and size as the other sparassid species (Fig. 4.16).

Embryos from *I. vaster* and *P. regina* possessed either 43 or 46 chromosomes, which suggests that they have a sex determining system of the $X_1X_2X_3/X_1X_1X_2X_2X_3X_3$ type.

(b) Meiosis.

Meiotic preparations were successfully obtained for all of the species shown in Table 4.1. Meiotic cells were most commonly encountered between the stages of leptotene and diakinesis, and occasionally at second anaphase (Figs 4.17, 4.18). First metaphase cells were rarely encountered, either because the technique for producing the chromosome spreads destroyed the spindle fibres, or because this stage is very rapid. No first anaphase cells were seen.

Early in meoitic prophase the nucleus takes on a granular appearance and the X-chromosomes condense and form a heavily staining cluster. In *I.* sp. 1, the condensation and clustering were not as pronounced as in the other species, and even at maximum contraction the X-chromosomes could be distinguished from one another (Fig. 4.21). In the other species the condensation was much greater, and the X-chromosomes formed an amorphous, heavily staining mass (Figs 4.22, 4.23). In later stages the X's had decondensed and dissociated slightly, and by diplotene they had similar proportions and staining properties to the autosomes in all of the species (Figs 4.24 - 35). Often, at diplotene and diakinesis, one of the X's appeared to be longer than the other two, but this was not consistent either between cells or individuals.

Normal chiasmate bivalents were formed in all of the species, and with one possible exception (Fig.4.32) a single terminal or interstitial chiasma was present on each (Figs 4.24 - 31, 4.33 - 35). From C-banded cells it was apparent that the majority of the terminal chiasmata were proximal to the centromere (Figs 4.24 - 27, 4.28 - 30, 4.35), except in *Heteropoda procera* where distal and interstitial chiasmata were more common. Chiasma distribution will be discussed in more detail later.

In the C-banded diplotene and diakinesis cells from *Olios* shown in Figures 4.29 and 4.30, the majority of chiasmata were proximal to the centromere, however in a gross stained early metaphase cell (Fig. 4.31) the morphology and alignment of the bivalents suggests that most were distal. Whether this is a reflection of genuine variation in the chiasma distribution pattern or simply reflects an aberrant cell is not clear.

From meiotic preparations it is apparent that heterozygosity for the distal C-bands commonly occurs, but because of the unreliability of the C-banding, the levels of heterozygosity cannot be estimated (Figs 4.25, 4.29).

4.1.3 Mitosis and meiosis in *Delena cancerides*.

Five distinct cytotypes or chromosomal races, differing in the presence or pattern of centric fusions were observed in this species. The geographic distribution of these is shown in Figure 4.78.

In all of these races three distinct forms of X-chromosome behaviour were observed during the early stages of meiosis:

(i) no differential contraction. In many meiotic prophase cells the X's could not be distinguished from the autosomes.

(ii) intermediate contraction with the X's terminally associated (Fig. 4.37). In cells of this sort, the X's were slightly more contracted than those of *Isopoda* sp. 1 (Fig. 4.21), and were always associated at one end, forming a tri-radiate configuration.

(iii) extreme contraction (Fig. 4.36), as in the majority of the other sparassid species described above.

These three degrees of contraction were often seen in meiotic prophase cells from the same animal, that were undoubtedly at the same

stage of meiosis. In some cases one of the X-chromosomes appeared to be longer, but as was the case in the other huntsman species, this did not occur consistently. Most commonly, no differential contraction was observed at meiotic prophase. By diplotene, the X's had usually assumed the same size and general morphology as the autosomes, though slight differences were still often observed in the intensity of their staining. By this stage, physical associations between the X-chromosomes had invariably lapsed. Even so, when segregation products were seen, the X's had always segregated to the same pole (Fig. 4.43). Rarely, the X-chromosomes did not decondense at all during the later stages of meiotic prophase and remained more heavily staining than the autosomes.

The five chromosomal races are described individually below.

(a) Twenty telocentric bivalents + three X-chromosomes (tII cytotype).

Specimens of this race were collected from Queensland, western Victoria and Tasmania (Fig. 4.78). Forty-three telocentric chromosomes were present in mitotic cells from males, and these were identical to those of the other huntsman species described above in size, morphology and C-banding pattern (Figs 4.38, 4.39). Meiosis was also indistinguishable from the other huntsman species described except that the X-chromosomes did not remain in a cluster, although they were usually found in close proximity to one another in the cell (Figs 4.40 - 42). At first anaphase the autosomes decondensed slightly, and the X's could often be distinguished by their smaller size and denser staining (Fig. 4.43). One of the anaphase I products consisted of twenty autosomal half-bivalents each with two chromatids each, and the other of twenty autosomes and the three X's.

(b) Ten metacentric bivalents + two X-chromosomes (mII cytotype).

In this race, from western Victoria, South Australia and Kangaroo Island (SA), 42 of the chromosomes in the diploid male set have undergone centric fusion, resulting in a mitotic complement of twenty-two metacentrics and one telocentric chromosome (Fig. 4.44). In preparations of spermatogonial mitosis, the X-chromosomes often stained more heavily, and the chromatids showed less separation than those of the autosomes. Thus it could be determined from mitotic preparations that the single telocentric chromosome was an X-chromosome, and that the other two X's had fused to each other.

Meiotic pairing resulted in the formation of ten metacentric bivalents (Figs 4.45, 4.46). The two X-chromosomes (one metacentric and one telocentric) were usually seen in close proximity, but not touching, at one side of the cell. No more than one chiasma was present between each pair of homologous arms in the bivalents, and since the chiasmata were generally proximal it was often impossible to ascertain whether or not two chiasmata were present in each bivalent, one on each side of the centromere (Fig. 4.45).

The X-chromosomes stained more darkly in first anaphase preparations and both migrated to the same pole (Fig. 4.47).

No cells were successfully C-banded in this race.

(c) Chain of three chromosomes, nine metacentric bivalents + metacentric X-chromosome (CIII).

In this population from Perth, Western Australia, wholesale fusion of the karyotype has also occurred, but here only nine of the fusions are present in the homozygous state. The other fusions involve two of the X-chromosomes (X-X fusion), an X-chromosome and an autosome (X-A fusion), and two autosomes (A-A fusion). The remaining, unfused chromosome is an autosome. Meiotic pairing results in the formation of

nine metacentric bivalents, a free metacentric X-chromosome, and a chain of three chromosomes terminated at one end by the telocentric autosome, and at the other by the X-arm of the X-A fusion product (Figs 4.48, 4.49). The chain of three chromosomes is formed by the pairing of the free telocentric autosome with one arm of the A-A fusion product, and the pairing of the autosomal arm of the X-A fusion product with the other arm of the A-A fusion product. One chiasma was present in each pair of homologous arms. The metacentric X-chromosome remained at the periphery of the meiotic cells and did not associate closely with the X-arm of the X-A fusion product. This chromosome was often lost during the production of chromosome spreads.

As was the case in the mII race, chiasma position in the free bivalents in the CIII race was most commonly proximal.

(d) Chain of five chromosomes, eight metacentric bivalents + metacentric X-chromosome (CV cytotype).

Wholesale fusion in this race has also resulted in a karyotype consisting of one telocentric and twenty-one metacentric chromosomes (Fig. 4.50), including an X-X fusion, an X-A fusion and a telocentric autosome (Fig. 4.51). It is clear that these chromosomes are the result of centric fusions because the centromeric C-bands in the presumed ancestral telocentric karyotype are still present, in addition to some telomeric C-bands.

From meiotic preparations it is apparent that this race, from the northern half of New South Wales, is heterozygous for four centric fusions. Consequently, the meiotic cells observed consisted of the metacentric X-chromosome, eight metacentric bivalents and a chain of five chromosomes (CV) (Figs 4.52 - 4.54). As in the CIII race, the CV was terminated by the telocentric autosome at one end and by the X-arm of the X-A fusion product at the other, and one chiasma was present on

each pair of homologous arms. No consistent marker was present on the chain, but the chiasma between the telocentric and the homologous arm on the next chromosome was never observed to be proximal.

(e) Chain of nine chromosomes, six metacentric bivalents + metacentric X-chromosome (CIX cytotype).

The mitotic chromosomes of this race were indistinguishable from those of the CV race (Figs 4.55, 4.56). Meiotic cells, however, consisted of a chain of nine chromosomes terminated by the X-arm of the X-A fusion at one end and the telocentric autosome at the other, together with six metacentric bivalents and the metacentric X-A fusion (Figs 4.57 - 60).

The chiasma between the third and fourth chromosomes of the chain (counting from the telocentric autosome end) was always terminal, and a secondary constriction was present on one or both of the homologous arms of the seventh and eighth chromosomes in the chain (Figs 4.57 - 60). The secondary constriction was never seen in mitotic cells (Figs 4.55, 4.56).

Two first metaphase cells were observed. In both of these, the chain had adopted a zig-zag configuration indicative of alternate segregation (Figs 4.61, 4.62).

In addition to these five chromosome races, five individuals from the Canberra suburbs possessed a mixture of metacentric and telocentric chromosomes, and one or more chains of variable length (Table 4.2). The simplest interpretation of this is that these individuals are hybrid derivatives originating from hybridisation between the southern telocentric race and the CIX race. This assumption is supported by electrophoretic data which will be discussed later.

4.2 SYNAPTONEMAL COMPLEXES

Preparations from the stages of late zygotene and pachytene in *Isopoda* species are shown in Figures 4.68 to 4.70. Figure 4.68 shows parallel alignment of the chromosomes at pachytene as was inferred from the C-band patterns (Figures 4.19,4.20). All of the bivalents have synapsed completely but the X-chromosomes are not visible. In Figure 4.69 the separate lateral elements are visible in the synaptonemal complexes, but the resolution is insufficient distinguish the central elements. One end of each complex usually has a slightly thicker and more darkly staining body. It becomes clear in later preparations involving metacentric chromosomes that this body is associated with the centromeric end of the complex. Two bivalents in this cell have not yet fully synapsed, with the regions distal to the centromere still unpaired. In Figure 4.70, three pale, unpaired elements are visible which are presumably associated with the X-chromosomes. These are associated at one end but there is no evidence of a junction lamina as reported as occurring between the X-chromosomes in wolf spiders (Benavente and Wettestein 1977).

Figure 4.71 shows a leptotene cell from the tII race of *D. cancerides*. In this cell (as in the five other preparations obtained at this stage of meiosis) the centromeres are all clustered to one side of the cell. This mirrors the C-band clustering observed in the chromosome preparations on Figures 4.19 and 4.20. Figure 4.72 shows a pachytene cell from the same race, fully paired. The finer elements associated with the X-chromosomes are not visible. In Figure 4.73, which depicts a cell from the same race, the three X-chromosome elements are visible, all associated by one end only.

Figure 4.74 shows a pachytene cell from the CV chromosome race of *D. cancerides*. The free bivalents have all completely paired, and a

star-shaped structure arising from the pairing in the fusion multiple and an association with the metacentric X-chromosome can also be discerned. This structure is magnified in Figure 4.75 along with a schematic representation. The four darker rays are formed by the paired arms of the autosomal components of the chain, and the three paler rays are formed by the X-arm of the X-A fusion and the two arms of the metacentric X-chromosome which has formed a centromeric association with the X-A fusion product. Figure 4.76 shows the equivalent pachytene structure from the CIX race.

Figure 4.77 shows a zygotene preparation from the CIX race. In this cell, about 50% of pairing has been completed. The free bivalents can all be discerned, and pairing is proceeding in a zipper-like fashion from the centromeres towards the telomeres. Thickened regions corresponding to the centromeres are visible on these, and from this it can be inferred that the thickened ends on the complexes in the telocentric races are also telomeres. The structure associated with the fusion complex has been broken, but it is apparent that here pairing commenced in the interstitial regions. The X-chromosome elements are not visible.

4.3 ANALYSES

4.3.1 Interspecific differences in C-band distribution.

As mentioned above, the detection of distal C-bands was very unreliable. In an attempt to detect interspecific differences in the number of chromosomes with distal C-bands, a number of cells which had been C-banded in the same batch, on the same day using the same chemicals and identical treatments were scored. These counts are given in Table 4.3. Clearly no reliable conclusions can be drawn from these

data, however they do suggest that *I. vaster* and *O. diana* may have a higher frequency of telomeric C-banding material than the *Pediana* species. Given the range of variation even within cells from the same individual, statistical analysis of these data was not considered worthwhile.

4.3.2 Chiasma position.

Chiasma distribution was analysed to determine whether it differed between the chain carrying races and the bivalent formers, and between the chains and bivalents within a race.

The positions of chiasmata were partitioned as follows:

(i) Distal. - chiasma occurs in the 25% of the chromosome arm farthest from the centromere.

(ii) Interstitial. - chiasma occurs in the middle 50% of the chromosome arm.

(iii) Proximal. - chiasma occurs in the 25% of the chromosome arm closest to the centromere.

In metacentric bivalents it was often not possible to determine whether there were two extremely proximal chiasmata, one on each side of the centromere, or only one (for example, see Fig. 4.46, 4.47). In these situations only one was scored. Although rare, this would tend to bias the data such that races with metacentric bivalents would appear to possess a lower proportion of proximal chiasmata than was in fact the case.

In some cases overlaps prevented every chiasma from being scored in a cell.

Chiasma distributions were compared using the 'heterogeneity *G*-test' of Sokal and Rohlf (1981). The *G* statistic approximates the χ^2 distribution, but this test requires fewer calculations than the

traditional χ^2 heterogeneity test. Furthermore, while a correction is available, it "need be done only when total sample size is small and the observed *G*-value is of marginal significance" (Sokal and Rohlf 1981, p744).

(a) Bivalent formers.

Table 4.4 shows the proportion of proximal, interstitial and distal chiasmata in seven huntsman species, *G. godeffroyi*, and three bivalent-forming populations of *D. cancerides*. No allowance was made for variation between individuals within a species or population, but in forty-eight of the forty-nine cells scored, proximal chiasmata outnumbered both interstitial and distal chiasmata. The one exception was the one cell scored for *Pediana* sp. 1, in which equal numbers of distal and proximal chiasmata were present. Since a random chiasma distribution would result in equal numbers of proximal and distal chiasmata and twice as many interstitial chiasmata, it is clear that the distributions observed are markedly non-random.

(b) Chains and bivalents.

Chiasma position was scored for the three chain bearing races both for the chains themselves and the associated bivalents. Each position on the chain was scored separately, starting from the end with the telocentric autosome. These data are shown in table in Table 4.5. In contrast with the bivalent forming races, the majority of chiasmata in the chains are not proximal. Indeed, in only two positions (position 2 in CIX and 4 in CV) were proximal chiasmata more common than distal chiasmata. Moreover, at positions 1 and 3 of the CIX, positions 1,2 and 3 of the CV and position 2 of the CIII the majority of cells had a distal chiasma. In the CIX and CV races the chiasmata on the free bivalents were also more often distal than

proximal or interstitial, however in the CIII race the majority of the chiasmata on the free bivalents were proximal.

In the CV race, the distribution of chiasmata in the chain differed significantly from that in the free bivalents, owing to lower frequencies of proximal and interstitial chiasmata in the chain, and consequently a higher frequency of distal chiasmata. The significant difference in chiasma distribution between the bivalents and the chain in the CIII population was a consequence of an increased frequency of distal and interstitial chiasmata in the chain at the expense of proximal chiasmata. In the CIX race, the chiasma distribution on the chains was not significantly different from that of the free bivalents, however as noted above, the number of proximal chiasmata scored for the free bivalents is likely to be an underestimate of the true frequency.

Table 4.6 shows the results of G-test comparisons of chiasma position between chains and bivalents from the five discernable chromosome races. The chiasma distribution in the chain-of-nine differs significantly from that of the chain-of-five, but neither differ significantly from the chain-of-three. The chain-of-three and both the bivalents and chains in the CV and CIX races show greater chiasma "distalisation" than the bivalents in the mII and CIII races. The chiasma distribution in the bivalents of the CIII race and those from the mII race did not differ significantly.

4.4 DISCUSSION

4.4.1 General.

With the exception of the *D. cancerides* races which carry fusions, the karyotypes of the species examined did not differ appreciably in morphology or behaviour from those of other spider species that have been examined (see Chapter 1). The chromosomes were all telocentric and multiple X's were present. The *Uliodon* species had the same autosome and sex chromosome number as six related clubionid species (Suzuki 1952, Datta and Chatterjee 1983), and while the 2n=18 + 2X for *G. godeffroyi* is lower than the 26 + 2X of the majority of lycosid spiders that have been examined, its chromosome number is shared with three other members of this family (Brum-Zorrilla and Postiglioni 1980). All fifty of the lycosid species for which data is available also have two X chromosomes.

Both X/XX and $X_1X_2/X_1X_1X_2X_2$ sex determining systems exist in the Thomisidae, and four of the twenty-three thomisid species that have been analysed share a chromosome complement of 2n=22 + 2X with *Diaea socialis*. *D. subaldulta*, the only other congener which has been analysed, has a complement of 2n=26 + 2X, however. No other thomisid species are known to possess microchromosomes.

None of the twelve drassid species for which chromosomal data has been reported show the 2n=26 + 2X of the *Rebilus* species, but the autosome number in this family ranges from twenty to twenty-eight, and X/XX and $X_1X_2/X_1X_1X_2X_2$ systems both occur.

Selenops radiatus shares a complement of 2n=26 + 3X with the two *S. australiensis* specimens from New South Wales (Sharma *et al.* 1959) which suggests that the 2n=26 + 2X condition of the Northern Territory specimen may be derived.

With the exception of the fusion races of *D. cancerides*, the $X_1X_2X_3/X_1X_1X_2X_2X_3X_3$ sex determining system most common in the Sparassidae was shared by all of the species examined here, and the autosomal counts of thirty-eight and forty have also been reported in other huntsman species (Table 1.3). The fusion races are clearly derived from the primitive 2n=40 + 3X condition. In huntsmen, the autosomal number of 2n=38 + 3X is confined to the genera *Heteropoda* and *Spariolensis* (Table 1.3). If the 2n=38 condition is derived from 2n=40, one would expect the reduction in chromosome number to be accompanied by an increase in the size of at least one pair of the remaining chromosomes, if the genetic material of the missing pair is maintained. Thus it seems reasonable to assume that the 2n=40 condition is the more recently derived of the two. Unfortunately, owing to the absence of any phylogenetic data for this family, this cannot be said with any certainty.

4.4.2 Chromosomal fusion and chain formation in *D. cancerides*.

On the basis of the chromosome morphology and C-band patterns, the metacentric chromosomes of *D. cancerides* are undoubtedly the result of centric fusion. The regular presence of proximal, interstitial and distal chiasmata at the same chain position in different cells indicates that for many or all of the chromosomes, whole arm homology remains. If it did not, a high level of sterility would result due to the production of duplication/deficiency products. In this respect, *D. cancerides* conforms to the all-or-nothing rule for chromosomal fusion in spiders as discussed in section 1.4.1. That is, in the races of *D. cancerides* where fusions have taken place, the maximum possible number of fusions have occurred and no further fusions are possible. Unlike other spider species that conform to this rule however, fusions of X-chromosomes *and* autosomes, and fusions of X-chromosomes *to* autosomes have occurred *D. cancerides*.

In the mII race no X-A fusion has occurred and the fusions are all present in the homozygous state, so no unusual meiotic behaviour was observed. Since the morphology of the chromosome arms is so uniform, it is possible that this race could be comprised of adjacent populations each homozygous for different fusions. This could be clarified by further collections within the range of this race, since hybrids between races with different fusions could easily be detected by meiotic analysis, since such hybrid individuals would possess free-floating ring multiples.

Although female cell divisions could not be obtained in *D. cancerides*, it is clear that the chain carrying races possess complex sex-linked fusion heterozygosity, similar in essence to the sex-linked translocation heterozygosity which has been described in termites and a number of other species (see section 1.6.2). This conclusion is drawn from the following observations:

(i) an X chromosome is present in all of the chains,

(ii) adjacent arms on the chain are homologous, and

(iii) within the chain carrying races, all of the males sampled carry chains.

The homology of adjacent arms in the chain means that for balanced gametes to form, every second chromosome in the chain must migrate to the same pole, the remaining group of chromosomes moving to the other. This means that one of the two groups will carry the X-chromosome, consequently giving rise to female determining sperm, while the other group which carries no X-chromosome, must give rise to male determining sperm. If the group that does not migrate with the X-chromosome (the "Y-group") was also present in females, along with a free X-chromosome, it would occur in half of her male offspring which, with the Y-group from their father, would be homozygous for this group and produce bivalents at meiosis. No bivalent forming males were seen

within the distribution of the chain races except in the Canberra and Adelaide areas where this is believed to be artificial. This will be discussed in more detail later in this chapter.

A system of permanent fusion heterozygosity in all individuals, similar to that of the plant genera *Oenothera* and *Isotoma* (James 1965, Cleland 1972) could occur in *D. cancerides* if the Y-complex possessed lethal genes which caused inviability in the homozygous condition and a free X-chromosome was present. This is unlikely however, owing to the high genetic load it would engender and because such systems, although common in some plant groups, have never been reported in animals.

Figure (4.79) shows a schematic representation of the chromosomal behaviour in the CIX race, and this can be extrapolated to describe the behaviour of the other two races. According to this representation, females are homozygous for the X-group which is comprised of chromosomes 1, 3, 5, 7 and 9 of the chain, while males are heterozygous for the X-group and the Y-group (chromosomes 2, 4, 6 and 8 of the chain). On the basis of the distribution of these two chromosome groups, this system behaves as an $X_{1-5}Y_{1-4}$ sex determining system. Other than the fused X-chromosome the chromosomes in the chains in *D. cancerides* are obviously not sex determining chromosomes since, unlike the situation in the chain carrying termites (Syren and Luykx 1981), the original X's remain complete, discrete entities, and in the ancestral bivalent forming race no Y-chromosome was necessary for sex determination. Rather, the original sex determining system has provided a basis for which the non-random segregation of these autosomes can be effected.

Robertsonian fusions can be considered to be a special kind of translocation, since a whole chromosome has been translocated onto another. Thus the sex-linked fusion heterozygosity of *D. cancerides* is

a subset of the more general sex-linked translocation heterozygosity of termites and other organisms. Hence in future discussion the term "sex-linked translocation heterozygosity" will be used when both systems are being considered together.

The fact that the secondary constriction and consistently terminal chiasma always occur in the same position on the chain-of-nine indicates that the same chromosomes are involved in this chain throughout the range sampled, but owing to the uniformity of chromosomal morphology it is not possible to determine whether the same chromosomes are present and in the same order in the three types of chain. The terminal chiasma at position 3 on the chain-of-nine is uninformative in this respect because no corresponding position exists in the two shorter chains. Furthermore, the secondary constriction on the chain-of-nine is not present on any of the chromosomes in the other two chain carrying races, and so its absence in the two other chains does not imply the absence of the corresponding two chromosomes from them.

An interesting possibilty is that the females of different chain carrying races could potentially carry the same fusions and so be able to reproduce successfully with males of either race. In the present case this is unlikely, since the secondary constriction is absent in the CV race, but if this is merely a regional polymorphism, gene flow could be taking place relatively unhindered. In such a situation, it would also be possible for CV and CIX males to coexist but breed true, since the Y-complexes could never recombine with each other, because they are never present together in the same individual.

4.4.3 Synaptonemal complexes.

The fact that synaptonemal complexes are formed along the full length of the autosomes in the CV race of *D. cancerides* suggests that the autosomes are indeed centric fusions and homology exists along the full length of the chromosome arms, although some examples are known where synaptonemal complexes form between nonhomologous sections of chromosomes (Gillies 1984). In the permanent translocation heterozygote *Rhoeo spathacea*, complexes only form at the telomeric ends of the translocated chromosomes, but again it is unclear whether this is related to lack of homology at the proximal ends or selection for distal chiasmata (Stack and Soulliere 1984). Similarly, in the grasshopper *Stethophyma grossum* which is a normal diploid, chiasmata form only in the proximal regions of some bivalents, and in these synaptonemal complex formation is also limited to this region (Fletcher 1977).

Pairing of the chromosomes involved in chain formation is initiated interstitially rather than proximally, as in the free bivalents. This may be because, if all of the chain chromosomes started pairing in the centromeric region, this area would become extremely cluttered, and the process would be hindered. Thus, the interstitial initiation may be an automatic result of chain formation, or have been selected for to ensure correct pairing without excessive tangling.

Of considerable interest is the clustering of the X-chromosomes at meiotic prophase in all of the huntsman species analysed. In the lycosid species *Lycosa malitiosa* which possesses two X-chromosomes, a structure similar to a synaptonemal complex, termed a junction lamina (JL), forms between the two X-chromosomes and is present from pachytene until diakinesis (Benavente and Wettstein 1977). The fact that the huntsman X-chromosomes do not form a similar structure may be

due to the fact that three are present instead of two. Benavente and Wettstein (1977) believe that the JL may have a role in ensuring correct cosegregation of the X-chromosomes at first anaphase, and it is suggested here that the clustering behaviour of the huntsman X-chromosomes may also be important in their segregation.

4.4.4 Distribution of the chain carrying races of *D. cancerides*.

In eastern Australia, the presence of tII cytotypes both north and south of the fusion races (Fig. 4.78) suggests that the ancestral 2n=40 + 3X race was firmly established in this area before the fusion races arose. That the fusion races all appear to have contiguous distributions is consistent with a common origin for all of them. Their extensive present-day distributions may also be interpreted as evidence that they are adaptively superior to the the primitive form and have displaced it. This will be discussed later. Note that the CIII individuals from Perth WA are not included in this figure.

There is no obvious climatic correlate for the distribution of the fusion races, since all were collected both from coastal and dry inland areas.

Repeated attempts to collect specimens from the change-over areas between the CV and CIX races and the CIX and tII races proved fruitless. Inexplicably, in the Hawkesbury River basin between the CIX and CV races no huntsman spiders of any species were found despite the presence of suitable host trees. Between the distributions of the tII and CIX races on the Victorian/NSW border extensive bushfires had destroyed all loose bark, and no huntsman spiders were present.

In three areas a mixture of racial types were found, but in each case there is strong evidence that this was the result of the artificial displacement of individuals (see Fig. 4.78).

The presumed hybrids collected in Canberra were from well established inner suburbs, so if immigrants had been brought in, there would be little chance of their chromosomes being 'diluted out' by invasions of the local CIX forms from outlying areas of natural bushland.

At Yellow Pinch National Park on the southern NSW coast, an individual male of the tII race was collected, but CIX individuals were collected to the north and south of this locality. Since this single individual was collected within 50m of a building site where large amounts of timber were stored it is probable that it had been brought in with these shipments. The fact that no hybrids were found in the vicinity is further evidence that it was a recent immigrant, particularly given that hybridisation can occur as indicated by the Canberra hybrid forms.

The five collection sites around the Adelaide area were all either in the city itself or from large eucalypts growing in close proximity to wineries, which are well known tourist destinations. Thus the presence of both CIX and mII forms is not considered to be evidence of a mixed natural population. In the nearest collections, from south-eastern SA and western Victoria, only mII or CIX individuals were collected; no mixed populations were found. It is unclear from the existing distributional data which of these two races may have been the original form in the Adelaide area.

4.4.5 The origin of sex-linked fusion heterozygosity in *D. cancerides*.

In Chapter 1 two models for the origin of translocation heterozygosity (sex-linked or otherwise) were mentioned - the successive inclusion of chromosomes into multiples via novel translocations, and the instant formation of multiples by the

hybridisation of two races each homozygous for different translocations. The applicability of these two models to the translocation heterozygosity of *D. cancerides* will now be discussed individually, along with a third model. All three models assume that some consistent driving force has acted on some populations of *D. cancerides* resulting in the present situation where all of the known populations have either completely telocentric karyotypes or wholly fused karyotypes, and no intermediate forms occur. The hybrid derivatives from Canberra are considered to result from human intervention, and have no relevance to the present discussion other than demonstrating that hybrids between these two races may be fertile.

Under this all-or-nothing fusion hypothesis, in karyotypes of the Ca + bII + 1X type seen in *D. cancerides*, "a", or chain length, will always be an odd number. This is because all fusion combinations that would lead to an even number of chromosomes in the chain will result in a remaining homologous pair of telocentric autosomes plus the telocentric at the end of the chain. Since fusion saturation must occur and homologues clearly may not fuse to each other, the chain-terminating telocentric chromosome will fuse to one of the homologues, bringing them both into the chain and producing a chain consisting of an uneven number of chromosomes. Using these logical steps, it can be seen that whenever one X-chromosome is fused to an autosome, a minimal chain-of-three will be present at meiosis. This is because if the X-A product simply paired with the telocentric autosomal homologue, the resulting structure would, in effect, be a chain-of-two - that is "a" would be equal to two, and thus be even. The CIII race, then, possesses the simplest meiotic configuration possible for a karyotype of *D. cancerides* that contains an X-A fusion.

In the discussion that follows, three models for the origin of translocation heterozygosity are dealt with individually, but it should be stressed that they are not mutually exclusive.

(a) Successive Inclusion (SI).

According to this model, the chromosome chains in *D. cancerides* have been gradually built up by the successive inclusion of more chromosomes into the chains. If this is correct, the CIII and CIV races may be intermediate forms the CIX race.

The inclusion of the chromosomes may have occurred in two ways:

SI(i). In the ancestral telocentric race, an autosome fused with an X-chromosome. The homologue of the fused autosome would then behave as a telocentric neo Y-chromosome, since it must segregate from the X-A fusion if balanced gametes are to result. Fusion of another telocentric autosome to the neo Y-chromosome resulted in the neo Y-chromosome becoming metacentric, and the homologue of the newly fused autosome becoming a neo X-chromosome, owing to the necessity for it to segregate from the homologous section of the neo Y. At this stage the chain would have consisted of three chromosomes - a telocentric neo X-chromosome, a metacentric neo Y-chromosome, and the X-A fusion - and would have been indistinguishable from the chain-of-three in the CIII race. Given the propensity of the chromosomes to fuse in this hypothetical population, additional fusions which were not involved with the chain would also have regularly occurred. With inbreeding, these fusions would have become fixed in the homozygous state, and once all of the free chromosomes became fixed in this way chain growth stopped because no more telocentrics were available for incorporation into the chain via fusion. In other populations more chromosomes were incorporated into the chain before "fusion saturation" occurred, resulting in higher order chains.

Random fusion in the free bivalents may have resulted in floating polymorphism and hence unlinked chains and rings, but these gradually drifted to homozygosity.

Further X-A fusions involving the other two X's did not occur. Possible reasons for this are presented below, and will be discussed in some detail, because they are also relevant to the two other origin models.

a) if another X were incorporated into the chain via fusion, half of the time this second X-A fusion product would be separated from the other X-A by an even number of chromosomes. Consequently balanced gametes could not be formed since, with alternate segregation, the two X's would have to migrate to opposite poles and infertility would result. Even if an odd number of chromosomes was present between the two X's, mechanical difficulties might be expceted to arise when X-clustering occurred at prophase because the two fused autosomes would also be brought into the cluster. Assuming that clustering is important in ensuring that the X's co-migrate, if its prevention caused random segregation only 50% of the sperm would be genetically balanced.

b) if another X-chromosome fused with an autosome not associated with the chain, clustering may also be inhibited for the same reason. Furthermore, at fusion saturation, three telocentrics would remain - the third unfused X-chromosome, the terminal autosome of the chain, and the homologue of the autosome that had fused to the second X-chromosome. Given the all-or-nothing rule, three alternatives are possible. Firstly, the two telocentric autosomes could fuse and thus the second X would be

incorporated into the chain. This would result in infertility unless there was an odd number of intervening chromosomes. Secondly, the third X-chromosome could fuse with the homologue of the autosome fused to the second X, and consequently these two X's would have to segregate to opposite poles, producing infertility. Thirdly, the third X-chromosome could fuse with the terminal telocentric autosome of the chain. Again, an uneven number of chromosomes must separate the X's, and X clustering may be inhibited.

SI(ii). The original stock from which the chain races evolved was already saturated with fusions in the homozygous state, and cytologically identical to the mII race. A fission then occurred in one of the autosomes, followed by a fusion of one of the two telocentric products to the telocentric X-chromosome. This would have resulted in a chain of three chromosomes consisting of the X-A fusion product, paired by its autosomal arm with the homologous arm of the fused autosome, and the telocentric fission product, paired with the other arm of the fused autosome.

Successive fissions of free autosomes and subsequent refusion of one fission product to the terminal telocentric autosome of the chain would have resulted in a stepwise increase in chain length by two chromosomes each time. The products of fission and refusion of the metacentric X-chromosome would be selectively eliminated for the reasons given in the SI(i) description.

If the SI model is a correct representation of the processes that were involved in the evolution of the chain races, then chain build-up is a dynamic process which may still be taking place.

(b) Hybridisation between races carrying different fusions.

According to this model, the chain races arose via hybridisation between two races homozygous for different fusions; one homozygous for the chromosomes in the Y-complex and the other homozygous for those in the X-complex. The race homozygous for the chromosomes in the X-complex must have possessed the X-A fusion that is present in the X-complex. Consequently, as explained above, the all-or-nothing rule of chromosomal fusion dictates that this race must itself have carried a chain of at least three chromosomes which must have arisen by a mechanism other than hybridisation, such as the SI model described above, or the CPF model which will be outlined below. Thus the two theoretical parental races were indistinguishable from the extant CIII and mII races already described. It is envisaged that this hybridisation occurred along a zone of contact between the two races, and the resulting chain-carrying race then spread to partially or totally replace the parental types.

Figure (4.80) shows the relevant chromosomes in the karyotpes of the two parental races (the X-X fusion and the other autosomes have not been included). The karyotype of the race carrying the X-complex has been designated "AA" rather than "XX" since, although this race has two X-complexes, males possess only one X-chromosome. Females in the AA race will be referred to as A^XA^X and males A^XA. Similarly the race homozygous for the Y-complex will be referred to as "BB".

The karyotype of the AA females is morphologically indistinguishable from that of the females of the chain carrying races (Fig. 4.79), and so all that is required in the hybridisation model is that hybrid males be generated and replace the parental AA males in the AA race. Furthermore, the AA race could be parental to both the CV and CIX races, and if this were the case, both races could have chromosomally identical females. Thus, at first sight this model

appears simple; this is not the case however. Three different products are produced by hybridisation of these two races, of which only one can survive if a stable, higher order chain carrying race is to result. These three hybrid products are illustrated in Figure 4.80. Firstly, hybrid females from both reciprocal crosses will possess both the X- and Y-complexes but two X's, and are designated A^XB^X. This means that they could potentially pass on both complexes to their offspring, instead of only the A^X complex which is required in a stable higher order chain race. Males will be of two types, one from each of the reciprocal crosses - the required A^XB and AB^X. The latter carries a ring of chromosomes, but this will segregate independently of the free X-chromosome, and so both the A and the B haploid karyotypes may be passed on to offspring irrespective of their sex.

Three possible scenarios can be envisaged to explain the presence of only the A^XB males:

(i) the A genome is incompatible with one or all of the BB race's X-chromosomes.

(ii) only one or a few hybridisation events occurred, and the undesirable hybrids were lost by chance.

(iii) in AB^X males, the chain will not segregate successfully at meiosis unless associated with an X-chromosome, resulting in infertility, and the two X's from the AA and BB races will not segregate from each other and so fertility in hybrid females will be reduced. This is particularly likely if the X-X fusion in the two races is not the same. That is, in one race the X-X fusion product is comprised of, say, X_1 and X_2 and in the other X_1 and X_3. This would produce a chain-of-X's on the end of the chain. Alternatively, the X's may have diverged in the two races such that crossing over of homologues in females produces inviability. Unbalanced

survivorship or sterility in hybrids is not uncommom (see for example Hutter and Ashburner 1987).

Once formed, races possessing higher order chains could undergo hybridisation with other chromomal races, resulting in progeny with smaller or larger chains, or with chains of the same size but with the chromosomes occurring in a different order. For example, if this model is correct, the CV race may be parental to the CIX race or *vice versa*. This model will be explored in more detail in Chapter 6.

(c) Catastrophic Pan-Fusion. (CPF)

According to this model, an unknown factor caused spontaneous random fusion of the whole karyotype in *D. cancerides* over a large geographic range and a relatively short time period. No mechanism has ever been postulated for such a process, but this is because spiders are unique in their all-or-nothing fusion behaviour and it has never been discussed in the literature. Possible candidates for fusion agents may include:

(i) a transposable element activated by an environmental trigger. This element may have operated in one of the following ways:

(a) it may have coded for a gene product which caused chromosomal fusion.

(b) it may have inserted into a sequence present in all of the centromeres causing subsequent homologous pairing of this region at meiosis, crossing over, and hence fusion.

(c) an environmental factor may have caused the spontaneous excision of transposons already present in the centromeric regions leaving sticky ends which randomly fused.

(ii) a virus transported by a highly mobile prey species which integrates into the genome and causes fusion as in (b) above.

(iii) a chemical. Some chemical mutagen, perhaps a toxin, produced in the environment caused fusions specifically in this species. This may have been produced by a prey species or by the trees on which the colonies lived.

Once these fusions had occurred, the majority of fusion combinations resulted in sterility, due to the presence of more than one X-A fusion. Thus the population density was drastically reduced, resulting in abundant resources for the survivors, large areas to repopulate and reduced intraspecific competition - in fact a perfect situation for the operation of founder effects. Consequently, selective pressures were relaxed and even those survivors with slightly lowered fertility would have stood a high chance of survival and reproduction. Of the remaining fertile individuals, two types were present - those with one X-A fusion and an X-X fusion, and those with an X-X fusion and an unfused X-chromosome. Chance homozygosity for autosomal fusions would be expected to have been extremely rare, and so the vast majority of individuals would have formed ring multiples at meiosis, and those with an X-A fusion would have also possessed sex-linked chains.

In local populations where more than one X-A fusion was present one of these would gradually come to predominate, since if more than one were present two would regularly appear in females by virtue of their possessing two X-chromosomes. These females would show lowered fertility for the reasons given in the earlier discussion of the SI model, and hence females would act as a screening mechanism for removing multiple X-A fusions from the population.

Once population sizes began to increase and intraspecific competition again became important, selection would act to minimise heterozygosity for floating fusions in the population since, however

effective the segregation mechanism, the segregation of fusion multiples is still a more complex process than bivalent segregation and consequently more prone to error. Thus, populations with no X-A fusions would tend towards bivalent formation in the autosomal complement, and those with an X-A fusion would tend towards bivalent formation in the autosomes not involved in sex-linked translocation chains. Selection cannot remove fusion heterozygosity for the chromosomes involved in sex-linked chains since these are inextricably linked to the sex determining mechanism, and so the ability to segregate fusion multiples would also have been automatically selected for if no preadaptation for this was already present.

The final result of this process would be that within the geographical range where the CPF process had occurred, a mosaic of populations would exist, each carrying chains of different length or totally homozygous for chromosome fusions.

All of the above models, while plausible, suffer from inadequacies or rely on unproven and often unprovable assumptions. If SI(i) is correct and chromosomal fusion took place gradually, it is surprising that now only races with no fusions or wholesale fusions exist, and no intermediately-fused races races have been found. SI(ii) requires that fissions as well as fusions have occurred, but this has not been observed in spiders. Both of the SI models require the fixation and spread of successive chromosome fusions in the homozygous state in females and the heterozygous state in males. It is difficult to envisage how this could occur by chance, and so these models rely fairly heavily on a selective advantage in chain formation. As will be discussed in the next section, there is little evidence for this.

The hybridisation model is attractive because both of the parental types that it predicts are necessary for the production of a

chain bearing race do indeed exist, but there is no simple explanation for the presence of male hybrids only. While certain genetic incompatability effects may cause this, again it is difficult to see how the hybrid males could have displaced the ancestral AA males without assuming an adaptive advantage for the hybrids. The action of some other process for the initial production of the chain-of-three parent is also required for this model to effectively result in the production of a fixed, permanently sex-linked heterozygous population.

The CPF model is simple and explains the existence of all of the chromosome races, however there is no evidence for the spontaneous occurrence of any chromosomal rearrangement throughout a population. Nevertheless, the very fact that *D. cancerides* does consist of a number of different fusion races argues for a rapid fusion mechanism, since the probability of random generation and independent fixation of twenty-one fusions in four races and subsequent population expansion resulting in today's large geographic distributions is extremely low. Furthermore, this model does not rely on a selective advantage engendered by sex-linked fusion heterozygosity, and it would even be possible for the fusion heterozygote races to have a lower fitness than the ancestral, telocentric race.

In summary, all three models suggested here are possible but all suffer drawbacks. The CPF model is favoured since it does not require that complex sex-linked fusion heterozygosity have any selective advantage, however there is no evidence for spontaneous wholesale fusion having occurred in any other species. Whilst it is believed that these models cover all likely possibilities for the evolution of chromosome races, it is recognised that the three are not mutually exclusive.

4.4.6 Chiasma distribution and recombination.

The high frequency of extremely proximal chiasmata in most of the huntsman species examined here (Table 4.4) is unusual having only been reported once before in spiders, in species of the salticid genus *Pellenes* (Maddison 1982). Terminal chiasmata in spiders have often been reported in the literature, but since most of these were analysed before C-banding techniques were perfected and the camera-lucida drawings of first metaphase are often uninformative, it is not clear whether proximal chiasmata are generally common in spiders. In any case, neither proximal nor distal chiasmata cause marked genetic recombination. Proximal chiasmata merely result in the swapping of centromeres and perhaps a small amount of pericentric genetic material between two homologous chromatids, and distal chiasmata cause the recombination of a small amount of telomeric chromatin. Only interstitial chiasmata produce any major reshuffling of genetic material, and the highest observed incidence of these, in *I. tepperi*, was only 28% (Table 4.4). Thus it is a reasonable generalisation to say that recombination in male huntsman spiders is low.

In other species chromosomal fusion has been accompanied by a distalisation of the chiasmata (White and Chinnick 1957, Hewitt and John 1972, John and Freeman 1976, Shaw 1981). (The term "distalisation" is used here to mean an increased tendency to form distal chiasmata, and should not be confused with "terminalisation" which refers to the distal movement of chiasmata.) This is not the case in the *D. cancerides* mII race which is homozygous for chromosomal fusions, since in this race the chiasmata distribution does not differ significantly from that of the wholly telocentric tII race.

Conventional wisdom has it that translocation multiples possessing proximal chiasmata cannot segregate alternately, since proximal chiasmata reduce the flexibility of the mutiple such that it

cannot twist to form a zigzag configuration on the metaphase plate (Darlington and Gairdner 1937, Lewis and John 1963 p364, White 1973, Sybenga 1975 p212). While many of the translocation heterozygotes which have been studied, such as *Rhoeo spathecea* (Stack and Soulliere 1984), *Euchroma gigantea* (Mesa and Fontanetti 1984), *Paeonia* (Stebbins and Ellerton 1939), *Isotoma petraea* (James 1965), *Oenothera* (Cleland 1972), mistletoes (Barlow and Martin 1984, Barlow and Wiens 1976), termites (Syren and Luykx 1981, Vincke and Tilquin 1977) and cockroaches (John and Qraishi 1964) do have predominantly terminal and subterminal chiasmata, more recent work has cast some doubt on the traditional view that these are necessary for correct segregation (Rickards 1983). Most of the species listed above possess large proximal 'differential' segments (that is, segments between the centromere and the point of crossing over) and it may be that the main reason for their distal chiasmata is that there has been selection to prevent recombination within the differential regions. This is because these segments may often be nonhomologous, and crossing over in these regions would result in duplication/deficiency products.

The distribution of chiasmata in the chain carrying races of *D. cancerides* presents a paradox. Proximal chiasmata are not rare in these chains (for example, see Fig. 4.57 and Table 4.5) and so the traditional view cannot be correct in this case since the resulting infertility through nondisjunction would be prohibitively high. Even so, there has been a significant change to distal chiasmata in the chain races (Table 4.6). Two explanations for this may be:

(i) While alternate segregation can still occur in the presence of proximal chiasmata, these may cause occasional nondisjunction and so have been selected against.

(ii) There is a correlation between the point of synaptic initiation and chiasma position (Maguire 1974). If, as was

discussed in the last section, selection has favoured interstitial rather than proximal initiation of synaptonemal complex formation to prevent tangling of the complexes and mispairing, this may well have been passively accompanied by a shift in chiasma position.

Neither of these explanations is inconsistent with the fact that in both the CIII and CV races distal chiasmata are more common in the chains than the free bivalents. In the CV and CIX races however, the free bivalents also have a more distalised chiasma distribution than the bivalents in the CIII and mII races. This could be accounted for by the hybridisation model as follows:

According to the hybridisation model, the CIII race arose via the CPF or SI processes, and so has no hybrid ancestry. On the other hand, the CV and CIX races both have a hybrid ancestry with a CIII race as an ancestor, but one or both of their immediate parent races may have possessed longer chains. Thus some of the chromosomes that form the free bivalents in the CV and CIX races may themselves have been incorporated into chains at some earlier time and been exposed to selection for more distal chiasmata or interstitial pairing initiation, and this behaviour has remained despite the fact that they now form bivalents. The fact that the chiasma distribution in the chain-of-nine does not differ significantly from that of the free bivalents in the CIX race and that it shows less distal chiasmata than the chain-of-five may be attributed to the CIX race being of more recent origin than the CV race.

If the distalisation of chiasmata on the chain has resulted from selection, this implies that chiasmata position is selected independently on each chromosome, since in the chain-of-five and

chain-of-three the chiasma positions differ between the chains and the free bivalents. In the mistletoe species *Viscum fischeri, V. combretiocola, V. album* and *V. hildebrandtii* which carry ring multiples, a similar difference in chiasma position between the chromosomes in the translocation complex and those in the free bivalents occurs (Barlow and Martin 1984 and Barlow and Wiens 1976). This is apparent from the published figures but is not discussed by the authors. While it has been demonstrated that the segregation of translocation multiples in artificial hybrids of rye is under genetic control (Thompson 1956) and that correct segregation can be selected for (Lawrence 1958), it has not been shown whether this is related to chiasma position or some other factor.

There is no obvious reason why the chiasma at position 3 between chromosomes three and four on the chain-of-nine is always distal (Table 4.5). The effect of this is that the genetic material carried on chromosome three of the chain, which always segregates with the X-A fusion product, will always occur in the homozygous state in females, as does the X-A fusion product. The material on chromosome four of the chain will always and only occur in males, and always in the heterozygous state with the material of chromosome three. Since proximal chiasmata occur in all of the other positions on the chain-of-nine, only the genetic material in very close proximity to the centromeres on these chromosomes will be consistently sex-linked; the remaining material will be swapped between the complexes in every generation where proximal crossovers occur. In the chain-of-five and chain-of-three, no position consistently shows distal chiasmata (Table 4.5), and given the small sample size, there is no evidence that proximal chiasmata do not occur in all positions at least occasionally. Thus in all three of the chain carrying races, long-term sex-linkage of autosomal genetic material via sex-linked fusion

heterozygosity will be negligible. In the short-term however, some degree of sex linkage will occur since the majority of the chiasmata in the chains are interstitial or distal, and so the genetic material between the chiasmata and the centromeres in each complex will remain intact and linked until a proximal chiasma occurs in some later generation. Thus any argument for a selective advantage to complex sex-linked fusion heterozygosity must rely on this short-term effect only.

4.4.7 Selective advantages related to complex sex-linked fusion heterozygosity.

From Table 4.5 it is evident that, in the CIX race, the only material that is consistently sex-linked (and so could potentially be maintained permanently in the heterozygous state in males) is that carried on the homologous arms of chromosomes 3 and 4 in the chain, which always form distal chiasmata. This is only a small percentage of the genetic material involved in the chain, and it is improbable that selection has favoured the evolution of a sex-linked chain of nine chromosomes merely to effect the linkage of genetic material contained on two. In the short term, however, a larger proportion of genes are sex-linked for two reasons:

(i) distal and interstitial chiasmata combined occur more commonly than proximal chiasmata, and so, for example, if each arm of a metacentric chromosome in a chain carries an interstitial or distal chiasma, the proximal portions of the arms remain linked to one another. This is not the case in the primitive telocentric types where the segregation of the telocentrics with respect to one another is random.

(ii) since only one chiasma forms on each arm, only one of each pair of sister chromatids is recombined in each meiotic division.

Disregarding the sex-chromosomes, which are by definition sex-linked even in the telocentric races, the chain of nine chromosomes in the CIX race involves 40% of the chromosomal material. If no recombination occurred, like-sex offspring would share, on average, 60% of their genes. If proximal chiasmata formed at every chain position, average relatedness between like-sex sibs would be reduced to 55%. Given the frequency of chiasmata positions in Table 4.5, and assuming that interstitial chiasmata recombine exactly one half of the genetic material on a chromatid, like-sex sibs in the CIX population share, on average, 58% of their genes, and opposite-sex sibs share an average of 42% of their genes. Thus complex sex-linked fusion heterozygosity in the CIX race does result in an increase in relatedness (and hence uniformity) of like-sex offspring, but whether this represents a selective basis for the evolution of this chromosomal system or merely a side effect is unclear. In the CV race, the segregation of the chain and the chiasmata positions given in Table 4.5 will result in an average relatedness between like-sex sibs of 54.5%, and between opposite-sex sibs of 45.5%. In this case, the effect on relatedness is only slight, and the recombination pattern of only 10% of the genes is altered. Consequently, the effect of this chromosomal system on uniformity among offspring will be negligible.

These observations lend little support to my suggestion that the uniformity model of Luykx and Syren (1979) may also apply to *D. cancerides* (Rowell 1985, 1986), and I consider there to be little justification for maintaining this stance. Given that the effects of this chromosomal system are minimal, it would seem more reasonable to

assume that this chromosomal system did not evolve for any adaptive reason, but rather, that it is a chance phenomenon, or that its presence is the result of past selection for some other unrelated phenomenon.

Since no other adaptive reason for the presence of complex sex-linked fusion heterozygosity in *D. cancerides* is apparent (see Chapter 1), the CPF model is favoured here, because it does not require that there be any adaptive advantage in possessing this chromosomal system.

4.5 SUMMARY

All of the huntsman species examined here had three X-chromosomes and forty (*Isopoda, Delena, Olios* and *Pediana*) or thirty-eight (*Heteropoda*) autosomes. In addition to this karyotype, *D. cancerides* also possesses four distinct chromosome races which are characterised by the possession of wholesale karyotypic fusion. In one of these races (mII), the autosomal fusions are in the homozygous state and bivalents are formed at meiosis in both sexes. No X-autosome fusions are present. The other three races all possess a single X-autosome fusion and males are heterozygous for some of the autosomal fusions. In these races, pairing results in the formation of a chain multiple in the male, and presumably bivalent formation in the female. Three models which may explain the origin of these races were outlined; (i) gradual chain build-up caused by the fixation of successive fusions (SI), (ii) hybridisation between races homozygous for fusions, and (iii) the resolution of chains and bivalents after a catastrophic fusion event (CPF). An analysis of chiasma distribution indicates that the linkage of genetic material in the mII race and sex-linkage in the chain carrying races is negligible, and so the existence of any selective advantage related to the chromosome fusions is unlikely. For this reason the CPF model is considered to be the most plausible explanation for the origin of these races, since it explains the existence and widespread distribution of the races without requiring that the fusions confer any adaptive advantage on them.

SPECIES	NUMBER ANALYSED	2N (male)	2N (female)	LOCALITY
Isopoda villosa L. Koch	5	43	-	ACT, Duffholme (Vic)
I. vaster L. Koch	10	43	46	Captain's Flat (NSW), Kioloa (NSW), Bega (NSW), Lakes Entrance (Vic), Cann V. (Vic).
I. tepperi Hogg	12	43	-	ACT, Clare (SA), Tanunda (SA).
I. sp. 1	2	43	-	Melbourne (Vic).
I. sp. 2	1	43	-	Perth (WA).
Olios diana (L. Koch)	6	43	-	ACT.
O. sp. 1	1	43	-	Urapunea (NT).
Pediana regina (L.Koch)	42	43	46	Nyngan (NSW), Neara Ck (Qld)
P. sp. 1	1	43	-	ACT.
Heteropoda procera (L.Koch)	2	41	-	Brisbane (Qld).

Table 4.1 Collection sites and chromosome numbers for the species of *Isopoda, Olios, Pediana* and *Heteropoda* successfully karyotyped.

INDIVIDUAL	MITOSIS	MEIOSIS	
DC4	9m + 25t	CIX + 12II + X	-
DC5*	19m + 5t	-	Fig. 4.63
DC6	19m + 5t	2CV + CIII 5II + X	Fig. 4.64
DC7	5m + 33t	6CIII + 8II + 2X	Fig. 4.65
DC19	19m + 5t	CVII + 2CIII + 5II + X	Fig. 4.66

Table 4.2 Presumed interracial hybrids from the Canberra region in *Delena cancerides*. m = metacentric, t = telocentric.

*This individual was erroneously reported in Rowell (1985) as possessing 20m + 3t.

SPECIES	NO. OF INDIVIDUALS	NO. OF CELLS	MEAN	RANGE
Isopoda vaster	7	22	11.8	3 - 19
Pediana regina	1	4	2.5	0 - 5
Pediana sp. 1	1	2	3.0	2 - 4
Olios diana	2	4	8.25	7 - 10

Table 4.3 Number of telomeric C-bands visible in cells from four species banded under identical conditions.

SPECIES	PROX.	INTER.	DIST.	NO. OF ANIMALS	NO. OF CELLS
I. vaster	81%	11%	8%	5	22
I. villosa	87%	10%	3%	1	2
I. tepperi	51%	28%	21%	2	2
I. sp. 1	75%	10%	15%	1	1
O. diana	61%	17%	22%	2	3
P. regina	50%	10%	40%	1	1
P. sp. 1	40%	20%	40%	1	1
D. cancerides NtII	65%	22%	13%	3	3
D. cancerides StII	73%	20%	7%	3	3
D. cancerides mII	70%	12%	18%	6	6
G. goddefroyi	60%	22%	18%	2	5

Table 4.4 Percentage of proximal, distal and interstitial chiasmata in the huntsman genera *Isopoda, Olios, Pediana,* and *Delena,* and the lycosid *Geolycosa goddefroyi*. NtII = Northern telocentric race, StII = Southern telocentric race, mII = metacentric bivalent forming race.

CHAIN	CIX RACE			CV RACE			CIII RACE		
POSITION	P	I	D	P	I	D	P	I	D
1	5	2	12	0	1	9	1	2	2
2	5	11	3	1	0	8	0	1	4
3	0	0	19	0	2	8			
4	6	5	8	4	3	3			
5	5	8	6						
6	5	7	7						
7	2	10	7						
8	4	8	7						
TOTAL	32	51	68	5	6	28	1	3	6
%	21%	34%	45%	13%	15%	72%	10%	30%	60%
FREE	55	53	74	48	29	75	63	16	11
BIVALENTS	30%	29%	41%	32%	19%	49%	69%	18%	13%
G	3.556			7.636*			9.650**		

$\chi^2_{2,.05} = 5.991$, $\chi^2_{2,.01} = 9.210$

Table 4.5 Chiasma position in chains and free bivalents in the CIX, CV and CIII races *D. cancerides*. In each case, the chiasmata were scored from the telocentric end of the chain (position 1) to the X-A end. Using the G statistic, significant differences in chiasma position were observed between the chains and the free bivalents in the CV and CIII populations, but not in the CIX population. These significances are due to a tendency for chiasmata to be more proximal in the free bivalents and more distal in the chains. Sample sizes were: CIX (19 cells), CV (10 cells), and CIII (5 cells), however not every chiasma position could be determined in all cells.

		CIX		CV		CIII		StII
		chain	bivs	chain	bivs	chain	bivs	
CIX	b	3.556						
CV	c	9.332**						
CV	b			7.636*				
CIII	c	1.162		1.038				
CIII	b	59.670***	42.062***	50.200***	43.230***	9.650**		
mII		58.478***	40.270***	43.194***	36.740***	14.564***	1.862	5.022

$\chi^2_{2,.05}=5.991$, $\chi^2_{2,.01}=9.210$, $\chi^2_{2,.001}=13.816$.

Table 4.6 G-test comparisons of chiasma distributions in Delena chromosome races. Within the chain carrying races the free bivalents have been treated separately from the chains, since the chiasma distributions differ between these.

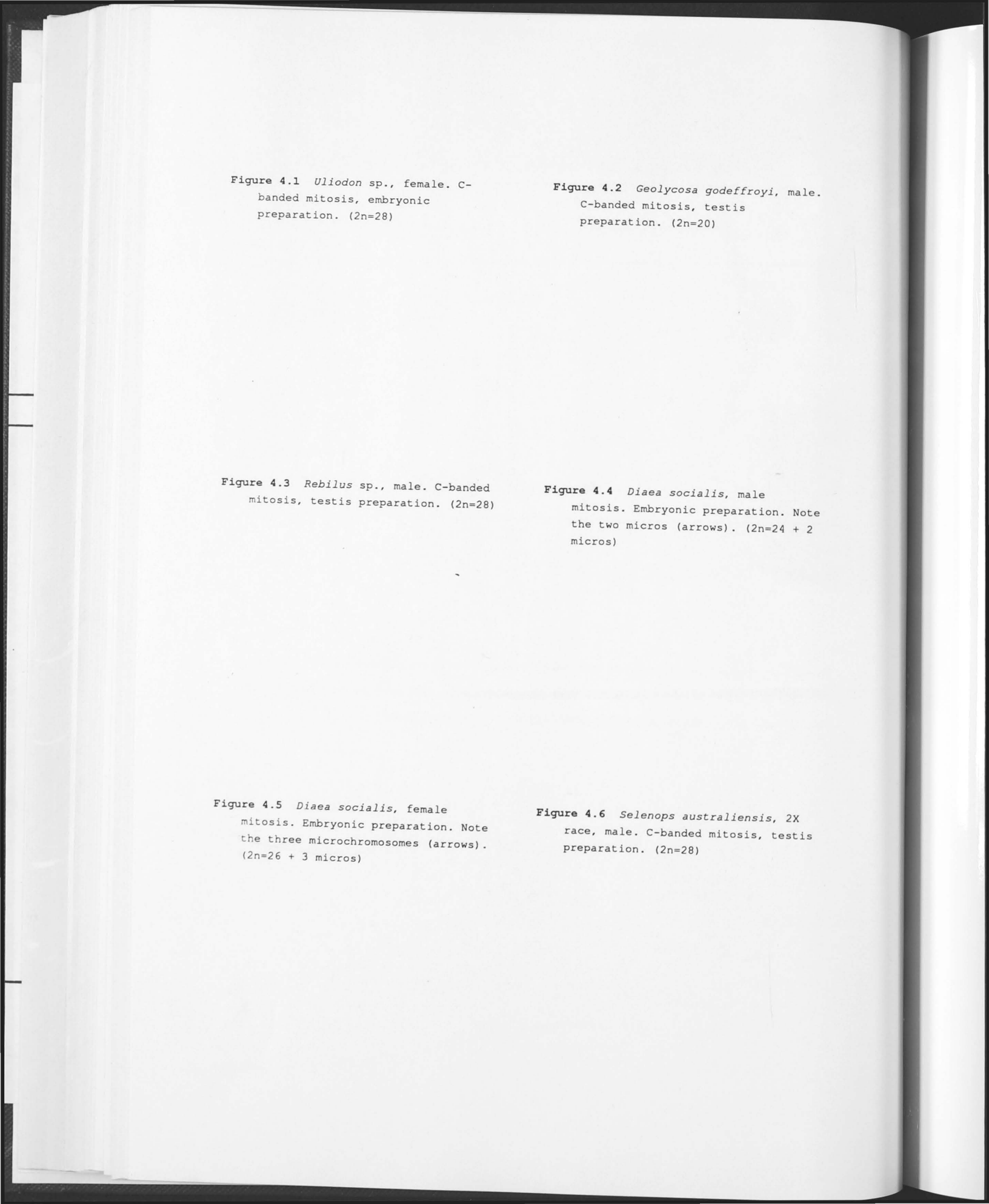


Figure 4.1 *Uliodon* sp., female. C-banded mitosis, embryonic preparation. (2n=28)

Figure 4.2 *Geolycosa godeffroyi*, male. C-banded mitosis, testis preparation. (2n=20)

Figure 4.3 *Rebilus* sp., male. C-banded mitosis, testis preparation. (2n=28)

Figure 4.4 *Diaea socialis*, male mitosis. Embryonic preparation. Note the two micros (arrows). (2n=24 + 2 micros)

Figure 4.5 *Diaea socialis*, female mitosis. Embryonic preparation. Note the three microchromosomes (arrows). (2n=26 + 3 micros)

Figure 4.6 *Selenops australiensis*, 2X race, male. C-banded mitosis, testis preparation. (2n=28)

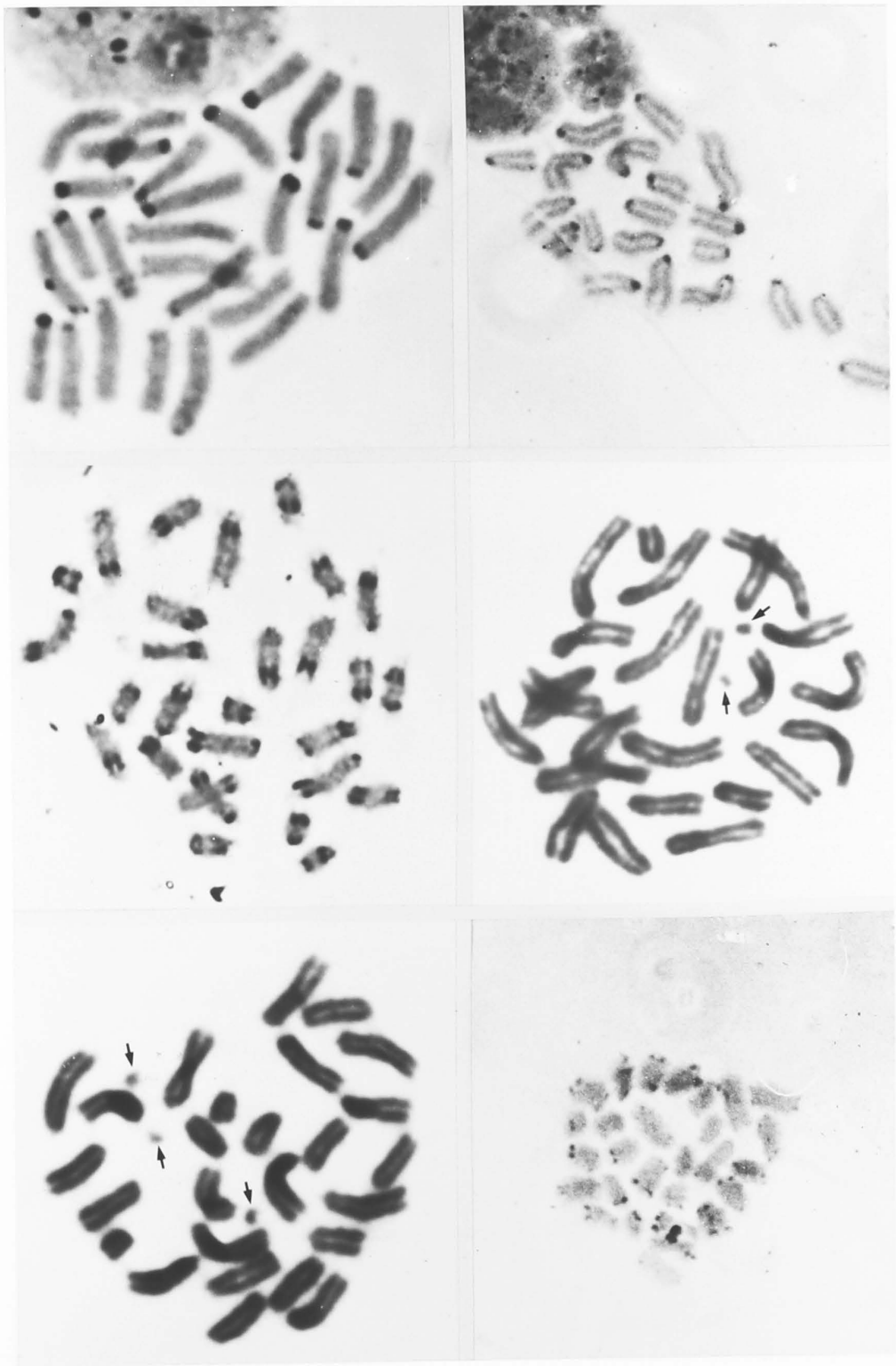


Figure 4.7 *Geolycosa godeffroyi*, C-banded male meiosis, diplotene/diakinesis. 9 bivalents + 2 X-chromosomes (arrows).

Figure 4.8 *Rebilus*, male, C-banded meiosis, diplotene. 13 bivalents + 2 X-chromosomes (arrows).

Figure 4.9 *Selenops australiensis*, 2X race. Male meiosis, diplotene/diakinesis. 13 bivalents + 2 X-chromosomes (arrows).

Figure 4.10 *Selenops australiensis*, 3X race. Male meiosis, diplotene/diakinesis. 13 bivalents + 3 X-chromosomes (arrow).

Figure 4.11 *Isopoda vaster*, female. C-banded embryonic mitosis. (2n=46) Kioloa NSW.

Figure 4.12 *Isopoda vaster*, spermatogonial mitosis. In spermatogonial preparations the two chromatids are often visible, as in this case. In embryonic preparations the chromatids always remain closely associated and cannot be distinguished (see Figs 4.11, 4.13). (2n=43). Kioloa NSW.

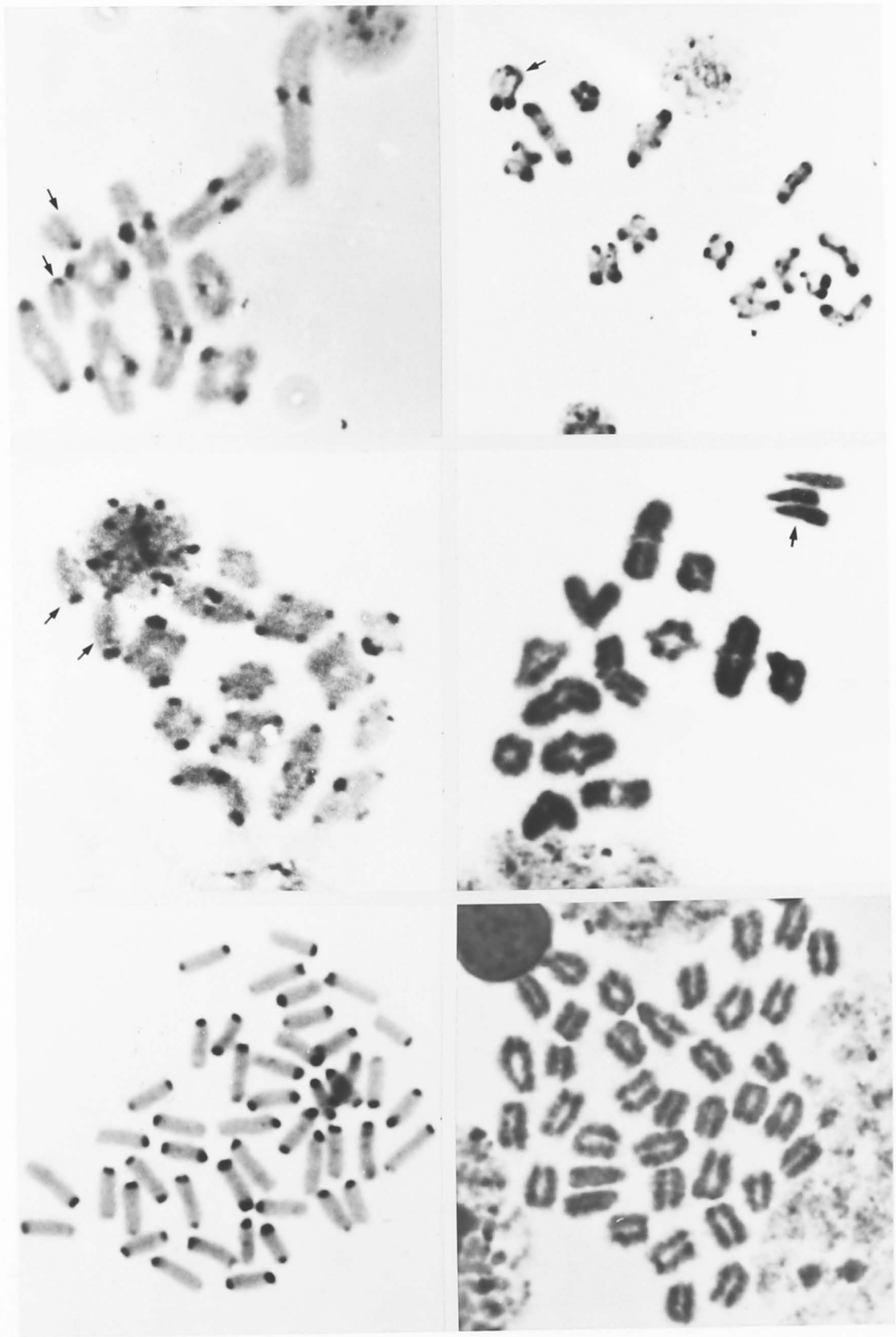


Figure 4.13 *Isopoda vaster*, male. C-banded embryonic mitosis. (2n=43). Kioloa NSW.

Figure 4.14 *Pediana regina*, male. C-banded embryonic mitosis. (2n=43). Neara Ck Qld.

Figure 4.15 *Olios diana*, C-banded spermatogonial mitosis. (2n=43). Canberra ACT.

Figure 4.16 *Heteropoda procera*, C-banded spermatogonial mitosis (2n=41). Brisbane Qld.

Figure 4.17 *Isopoda vaster*, C-banded male meiosis, anaphase II. Note that C-band positive material is present on every centromere. Each of the four products is haploid. The two products which will form male-determining sperm have no X-chromosomes and consequently n=20. In addition to the 20 autosomes, the other two products also possess the three X-chromosomes. Kioloa NSW.

Figure 4.18 *Isopoda vaster*, C-banded male meiosis, anaphase II. As in Figure 4.17, there is C-band positive material on all centromeres, and meiotic products of n=20 and 23. Captain's Flat NSW.

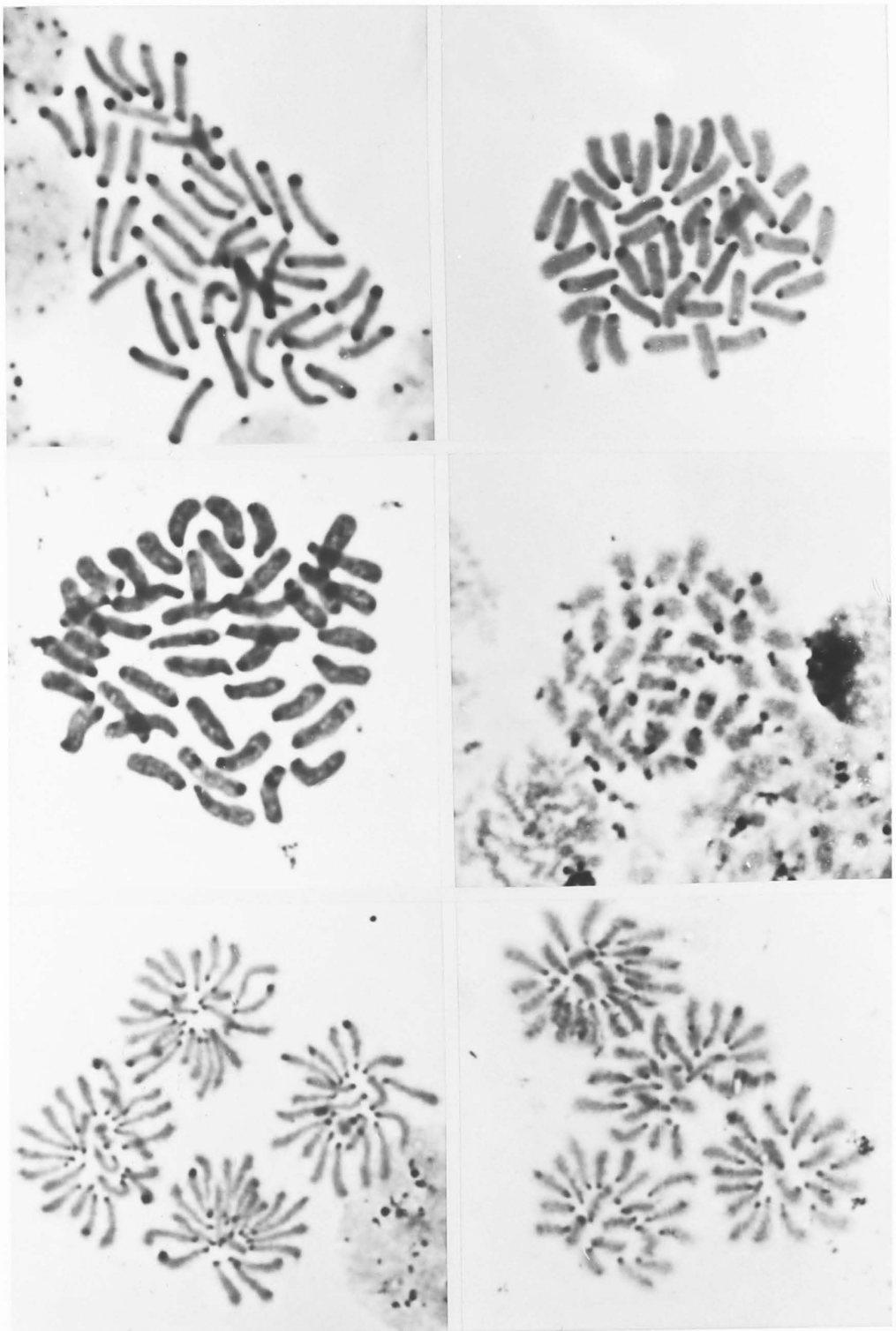


Figure 4.19 *Olios diana*, male. C-banded mitotic prophase. Note the two clusters of C-band positive material at opposite ends of the cell. The proportions of this material in the two groups are consistent with one group being centromeric and the other telomeric. Canberra ACT.

Figure 4.20 *Olios diana*, C-banded male mitotic prophase. Note the clustering of C-band positive material as in Figure 4.19. Canberra ACT.

Figure 4.21 *Isopoda* sp. 1, male meiotic prophase. In this species, the X-chromosomes show greater condensation and staining intensity than the autosomes at this stage of meiosis, but although the three are generally in close proximity, all three can be distinguished (arrows). Melbourne Vic.

Figure 4.22 *Olios diana*, male meiotic prophase. In all of the huntsman species examined, other than *Isopoda* sp. 1 (Fig. 4.21) and *Delena cancerides*, the X-chromosomes show extreme condensation at this stage, and form an amorphous, heavily-staining mass (arrows). In gross stained preparations such as this it is not possible to distinguish the three separate chromosomes. Canberra ACT.

Figure 4.23 *Heteropoda procera*, C-banded male meiotic prophase. In gross stained preparations from all of the species examined except *D. cancerides* and *Isopoda* sp. 1, the individual X-chromosomes cannot generally be distinguished as separate entities (Fig. 4.22). However, in C-banded preparations it is often possible to identify the centromeric regions of the three (arrow). Brisbane Qld.

Figure 4.24 *Isopoda vaster*, C-banded male meiosis, diplotene/diakinesis. 20 bivalents + 3 X-chromosomes. In this genus, the majority of the bivalents possess a single proximal chiasma. The X-chromosomes (arrow) are less condensed than in meiotic prophase and remain aligned until late in first division. Kioloa NSW.

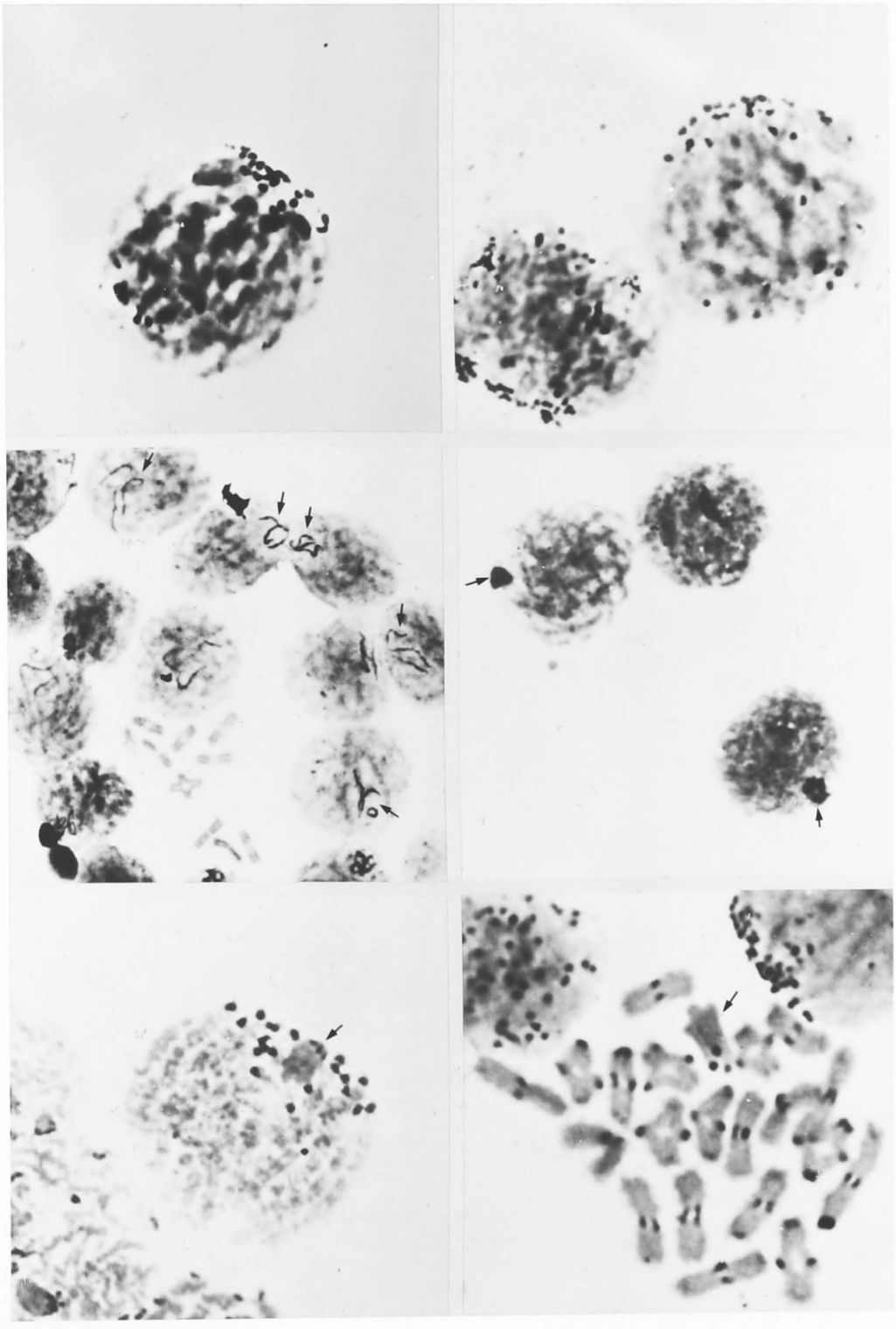


Figure 4.25 *Isopoda vaster*, C-banded male meiosis, diplotene. 20 bivalents + 3 X-chromosomes (arrow). * = bivalents heterozygous for telomeric C-banding material. Kioloa NSW.

Figure 4.26 *Isopoda vaster*, C-banded male meiosis. 20 bivalents + 3 X-chromosomes (arrow). * = bivalents heterozygous for telomeric C-banding material. Kioloa NSW.

Figure 4.27 *Isopoda vaster*, C-banded male meiosis. 20 bivalents + 3 X-chromosomes (arrow). Kioloa NSW.

Figure 4.28 *Isopoda immanis*, male meiosis, diplotene/diakinesis. 20 bivalents + 3 X-chromosomes (arrow). Atherton Qld.

Figure 4.29 *Olios diana*, C-banded male meiosis, diplotene. 20 bivalents + 3 X-chromosomes (arrow). Canberra ACT.

Figure 4.30 *Olios diana*, C-banded male meiosis, diakinesis. The X-chromosomes are missing from this preparation. Canberra ACT.

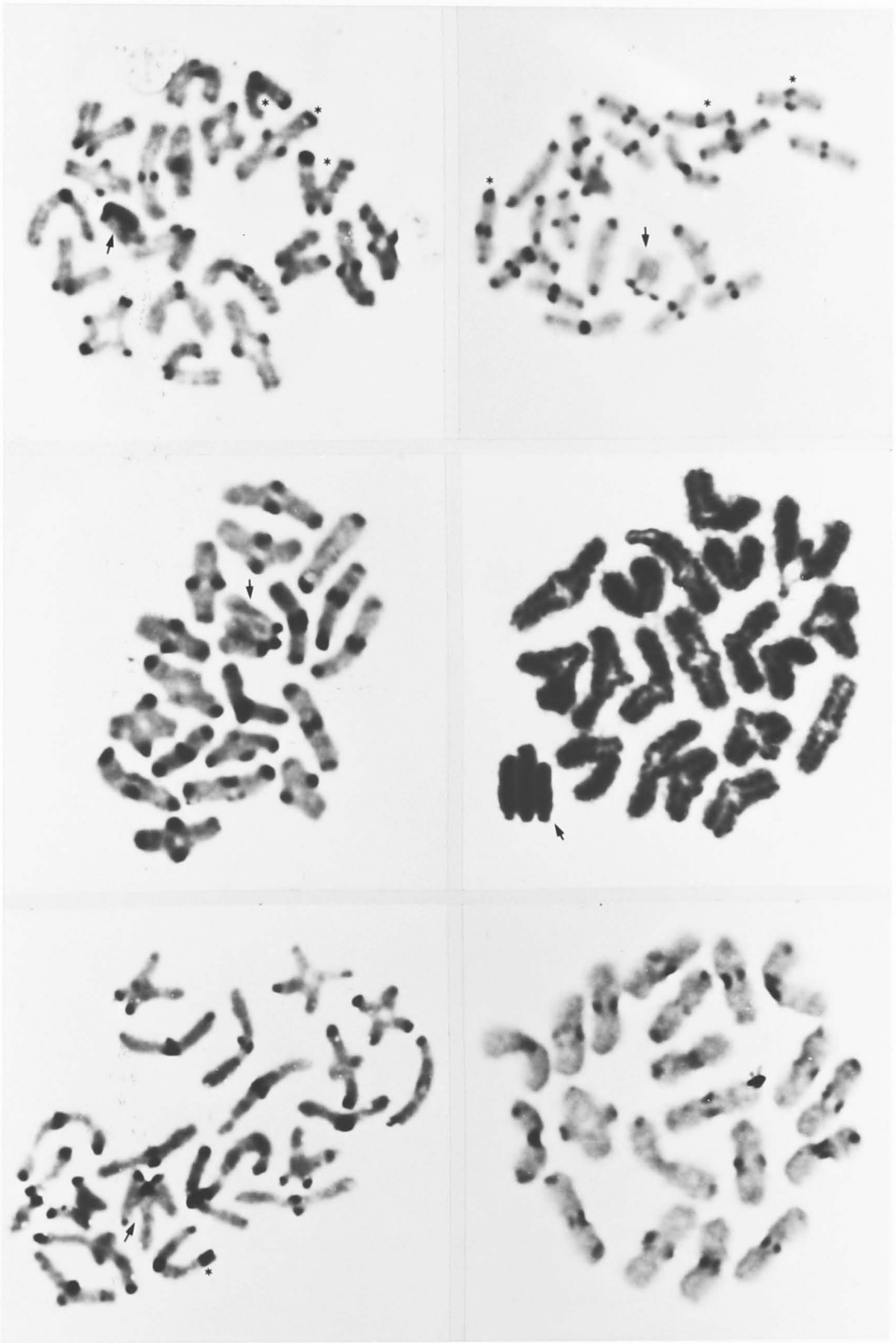


Figure 4.31 *Olios diana*, male meiosis, early metaphase I. Many of the bivalents in this cell appear to possess a distal chiasma, while in another cell from the same individual (Fig. 4.29) and in the cell shown in Figure 4.30, the majority of chiasmata are proximal. The X-chromosomes are missing from this preparation. Canberra ACT.

Figure 4.32 *Heteropoda procera*, C-banded male meiosis, diplotene. In this cell the majority of chiasmata are interstitial and, in two of the bivalents (arrows), there appear to be two chiasmata - one proximal and the other sub-distal. Brisbane Qld.

Figure 4.33 *Heteropoda procera*, male meiosis, metaphase I. 19 bivalents + 3 X-chromosomes (arrow). In this cell, the general morphology and alignment of the bivalents indicates that the majority of terminal chiasmata are distal. Brisbane Qld.

Figure 4.34 *Heteropoda procera* male meiosis, weak C-banding. In this incomplete metaphase I cell, the orientation, morphology and C-band position (where visible) indicate that at least six of the bivalents (arrows) have a distal or sub-distal chiasma. Brisbane Qld.

Figure 4.35 *Pediana regina*, C-banded male meiosis. 20 bivalents + 3 X-chromosomes. The X-chromosome cluster is visible at the top of the figure. Neara Ck Qld.

Figure 4.36 *Delena cancerides*, male meiosis, early prophase. Note the extreme condensation of the X-chromosomes (arrows). CIX race, Araluen NSW.

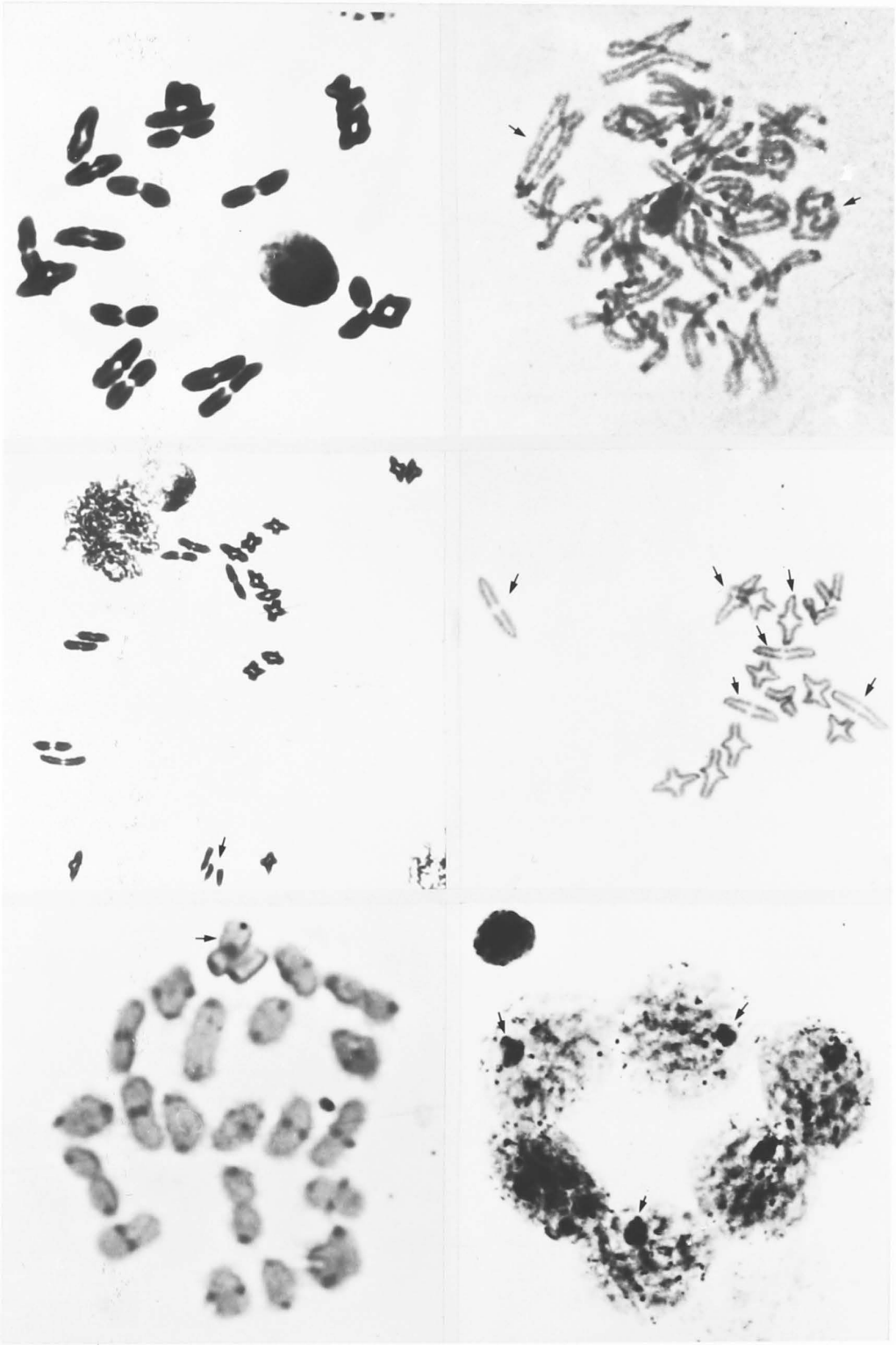


Figure 4.37 *D. cancerides*, male meiosis, prophase. X-chromosomes intermediately contracted and terminally associated (arrow). CV race, Tamworth NSW.

Figure 4.38 *D. cancerides*, C-banded mitosis, tII race (2n=43). Cherwell Ck Qld.

Figure 4.39 *D. cancerides*, male mitosis (2n=43). tII race, Cherwell Ck Qld.

Figure 4.40 *D. cancerides*, C-banded male meiosis, diplotene and diakinesis. 20 bivalents + 3 X-chromosomes (arrows). The X-chromosomes in this race are generally in close proximity to each other but do not remain in a cluster at meiosis. Cherwell Ck Qld.

Figure 4.41 *D. cancerides*, meiosis, diakinesis. 20 bivalents + 3 X-chromosomes (arrow). Cherwell Ck Qld.

Figure 4.42 *D. cancerides*, C-banded male meiosis, diplotene/diakinesis. One X-chromosome is missing from this preparation. 15km South of Biggendon Qld.

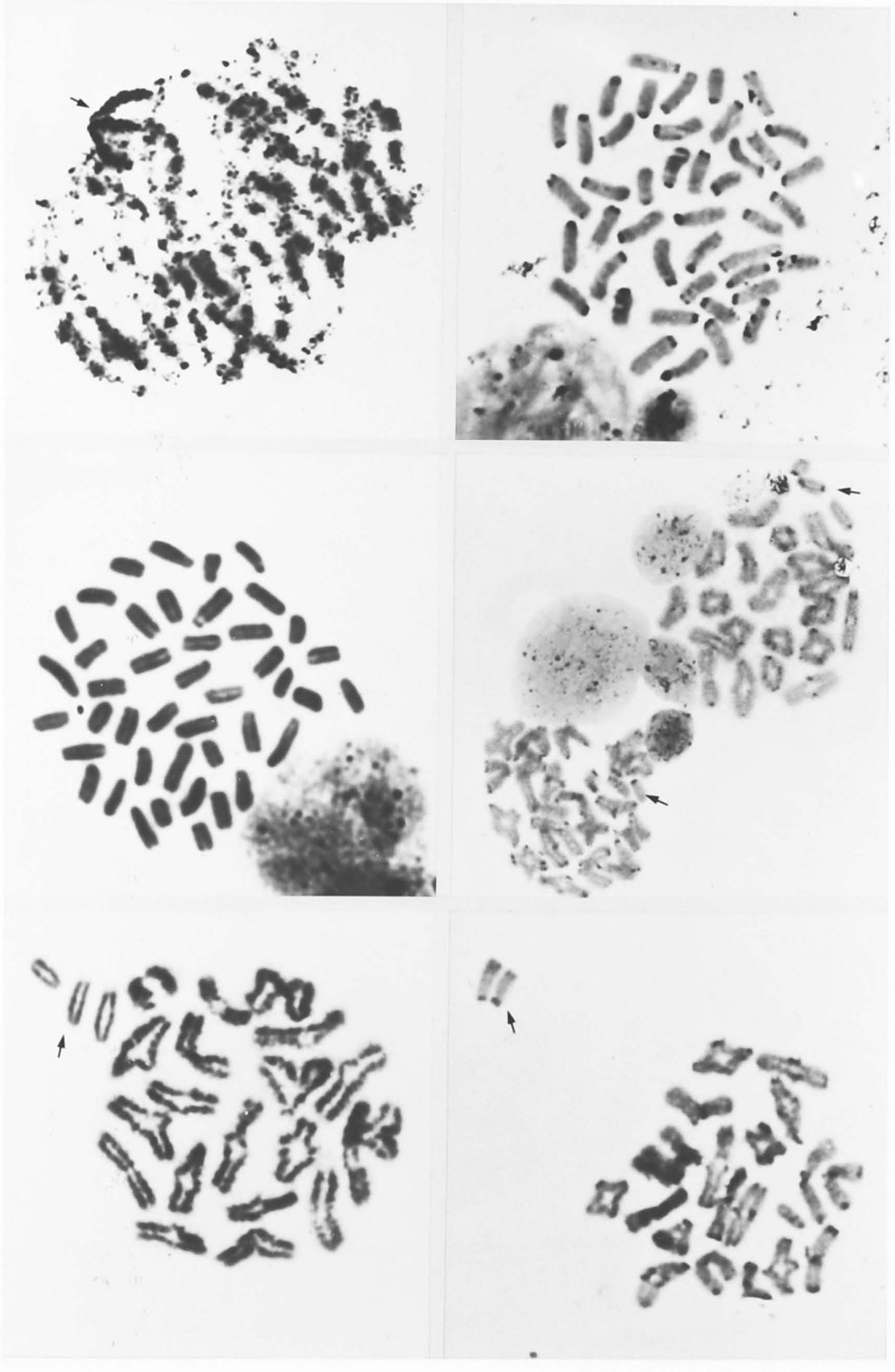


Figure 4.43 *D. cancerides*, male meiosis, anaphase I, tII race. The three telocentric X-chromosomes are visible in one of the two products, owing to their greater condensation (arrow). Cherwell Ck Qld.

Figure 4.44 *D. cancerides*, spermatogonial mitosis, mII race. The X-X fusion product can be distinguished by its more intense staining and the fact that the chromatids are less splayed apart than those of the autosomes. The telocentric X-chromosome is not obvious in this cell, but may be in the chromosome cluster to the right of the cell. Horsham Vic.

Figure 4.45 *D. cancerides*, male meiosis, diplotene, mII race. Ten metacentric bivalents are present, most commonly possessing one or two proximal chiasmata. The metacentric X-chromosome (m) and the telocentric X-chromosome (t) generally remain in close proximity, but without touching. Horsham Vic.

Figure 4.46 *D. cancerides*, male meiosis, diplotene, mII race. m = metacentric X-chromosome, t = telocentric X-chromosome. Glenthompson Vic.

Figure 4.47 *D. cancerides*, male meiosis, anaphase I, mII race. The two X-chromosomes (m & t) are more condensed than the autosomes at this stage. Their behaviour is very similar to that of the tII race's X-chromosomes (Fig. 4.43). Hall's Gap Vic.

Figure 4.48 *D. cancerides*, male meiosis, diakinesis, CIII race. Chain of three chromosomes + nine metacentric bivalents. The chain of three chromosomes terminates at one end with the telocentric autosome (t) and at the other with the X-arm of the X-A fusion product (x). The metacentric X-chromosome is missing from this cell. Perth WA.

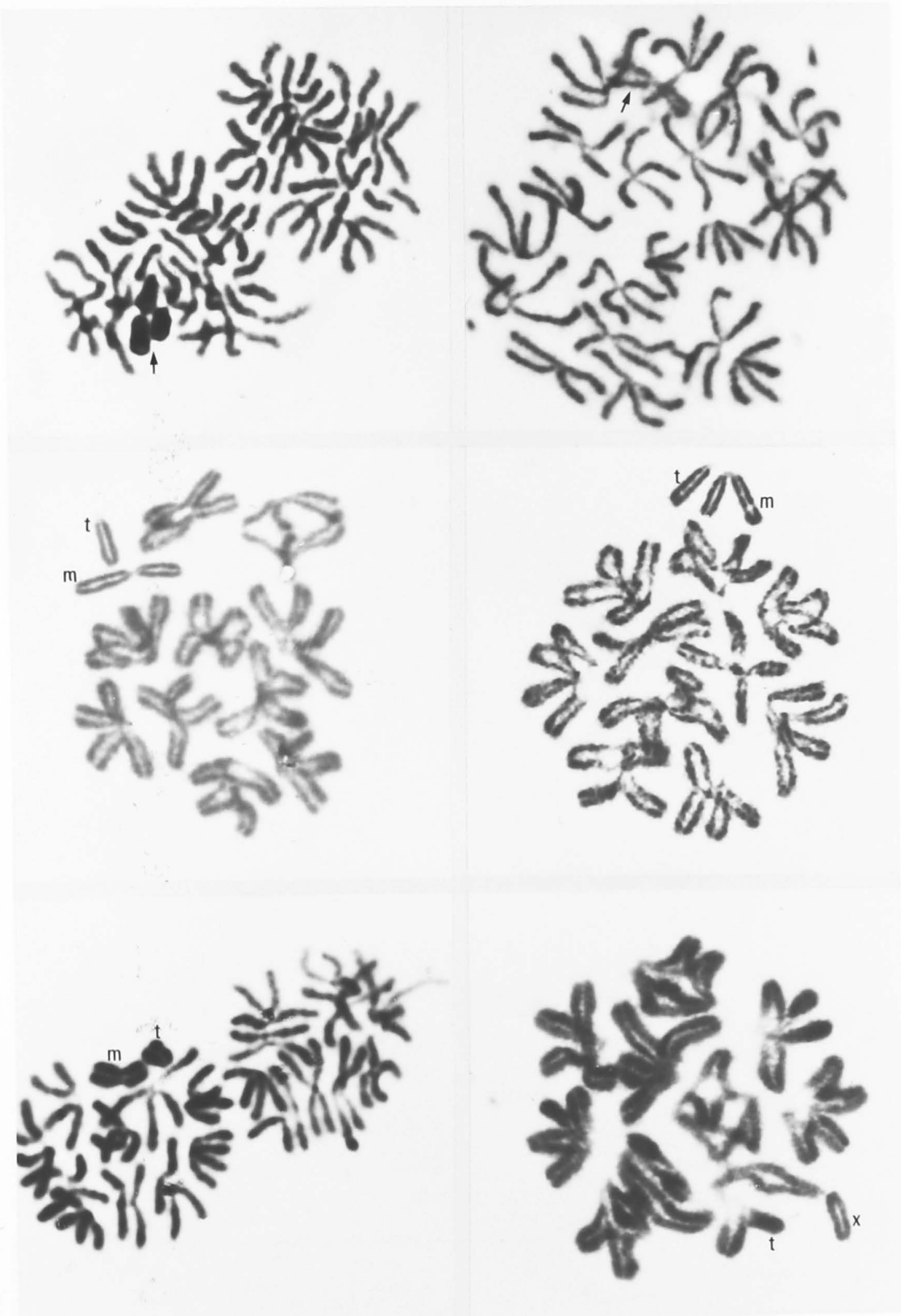

t
m
t
m
m
t
t
x

Figure 4.49 *D. cancerides*, male meiosis, diplotene/diakinesis. Chain of three chromosomes + 9 metacentric bivalents. The chain is terminated by the telocentric autosome at one end (t) and the X-arm of the X-autosome fusion at the other (x). The metacentric X-chromosome is missing from this cell. Perth WA.

Figure 4.50 *D. cancerides*, C-banded spermatogonial mitosis, CV race. The single telocentric autosome is indicated (t). Tamworth NSW.

Figure 4.51 *D. cancerides*, C-banded spermatogonial mitosis, CV race, incomplete cell. The X-X fusion product and the X-arm of the X-A fusion are clearly visible, owing to their greater condensation and staining properties. The telocentric autosome is indicated (arrow). Glencoe NSW.

Figure 4.52 *D. cancerides*, male meiosis, diplotene, CV race. Chain of five chromosomes + eight metacentric bivalents. The X-X fusion product is missing from this preparation. Toukley NSW.

Figure 4.53 *D. cancerides*, male meiosis, diplotene/diakinesis, CV race. Chain of five chromosomes + eight metacentric bivalents. The chromosomes in the chain are numbered from the telocentric autosome (1) to the X-A fusion product (5). The X-arm of the X-A fusion product is indicated (x). The metacentric X-chromosome is missing from this preparation. Tamworth NSW.

Figure 4.54 *D. cancerides*, male meiosis, diplotene, CV race. In this cell, the X-X fusion product and the X-arm of the X-A fusion product have remained condensed and in close association (x). Tamworth NSW.


t
x
t
4
5
x
3
2
1
X
5
4
1
2
3

Figure 4.55 *D. cancerides*, C-banded spermatogonial mitosis, CIX race. 22 metacentric chromosomes + 1 telocentric chromosome. The arms of the metacentric chromosomes bear a strong resemblance to the ancestral telocentric chromosomes (Figs 4.11, 4.13 - 4.16, 4.38, 4.39), indicating that there has been little (if any) loss of chromosomal material in the formation of the metacentrics. t = telocentric chromosome. (2n=22). Kioloa NSW.

Figure 4.56 *D. cancerides*, C-banded spermatogonial mitosis, CIX race. The X-X fusion product and the X-arm of the X-A fusion (arrows) can be distinguished by their more intense staining and the fact that their chromatids are not splayed apart. t = telocentric. Kioloa NSW.

Figure 4.57 *D. cancerides*, male meiosis, CIX race, diplotene/diakinesis. CIX + 6II + 1X. The chromosomes constituting the chain of nine are numbered from the telocentric autosome (1) to the X-A fusion product (9). The X-arm of chromosome 9 is unpaired. The chiasma between chromosomes 3 and 4 of the chain (c) is consistently distal in this race, and a secondary constriction is present on one or both of the homologous arms of chromosomes 7 and 8 (arrow). The metacentric X-X fusion product (m) is usually unassociated with the X-arm on the chain at this stage. Note the high frequency of proximal chiasmata on the chain in this cell. Kuringai NSW.

Figure 4.58 *D. cancerides*, C-banded male meiosis, CIX race, diplotene/diakinesis. Description as for Fig. 4.57. Note the extremely proximal chiasmata between chromosomes 4 and 5, and 5 and 6.

Figure 4.59 *D. cancerides*, C-banded male meiosis, CIX race, diplotene. Symbols as for Fig. 4.57. Kioloa NSW.

Figure 4.60 *D. cancerides*, male meiosis, chain of nine chromosomes, CIX race. The distinctive characteristics of the chain of nine chromosomes are clearly visible in this figure. A secondary constriction (arrows) is visible on both of the homologous arms of chromosomes 7 and 8. The X-arm of the X-A fusion product (9) has stained less intensely than the autosomes in this preparation. Tomakin NSW.

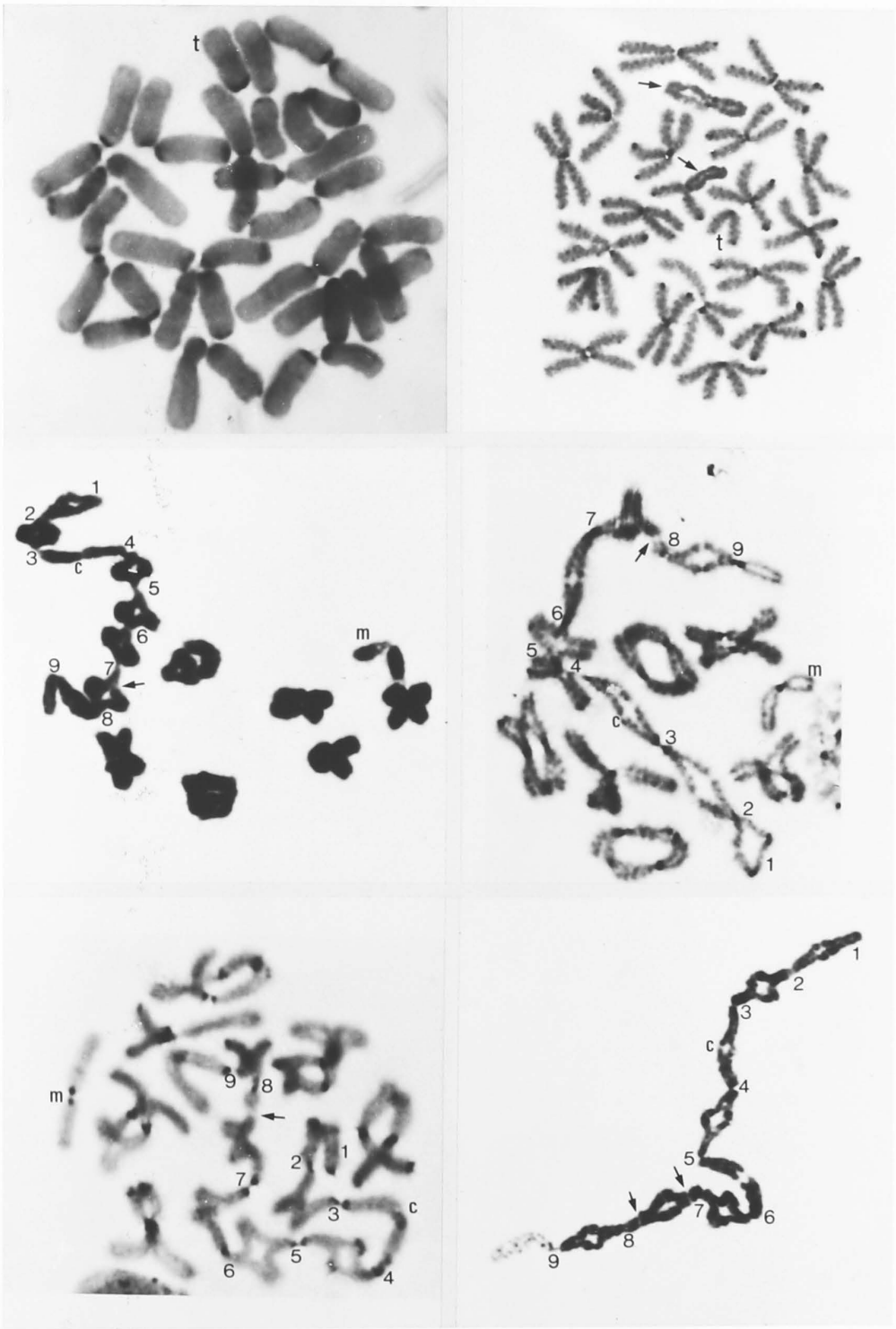

t
t
1
2
3
c
4
5
6
7
8
9
m
7
8
9
6
5
4
m
c
3
2
1
m
9
8
7
2
1
3
c
6
5
4
1
2
3
c
4
5
7
6
8
9

Figure 4.61 *D. cancerides*, male meiosis, CIX race, metaphase I. The zig-zag configuration of the chain indicates that alternate segregation of the chromosomes in the chain will occur at anaphase I. Kuringai NSW.

Figure 4.62 *D. cancerides*, male meiosis, CIX race, metaphase I. Note the zig-zag configuration of the chain. Araluen NSW.

Figure 4.63 *D. cancerides*, spermatogonial mitosis. This individual, presumed to be a hybrid derivative from the CIX and tII races, possesses 5 telocentric chromosomes (arrows) and 19 metacentrics. Canberra ACT.

Figure 4.64 *D. cancerides*, male meiosis, hybrid derivative, diakinesis. 2CV + CIII (arrow) + 5II + X (missing). The 2 chains of five have resulted from the replacement of the metacentric chromosome 5 in the chain of nine with two telocentrics. The original chromosome 9 of the chain (X-A fusion product) is situated directly above the arrow. Canberra ACT.

Figure 4.65 *D. cancerides*, male meiosis, diakinesis, hybrid derivative. 6CIII (arrows) + 8II + 2X. One of the 8 telocentric bivalents is missing in this preparation. The telocentric and metacentric univalents are the X-chromosomes. Canberra ACT.

Figure 4.66 *D. cancerides*, male meiosis, hybrid derivative, diakinesis. CVII + 2CIII (arrows) + 5II + X (m). Canberra ACT.

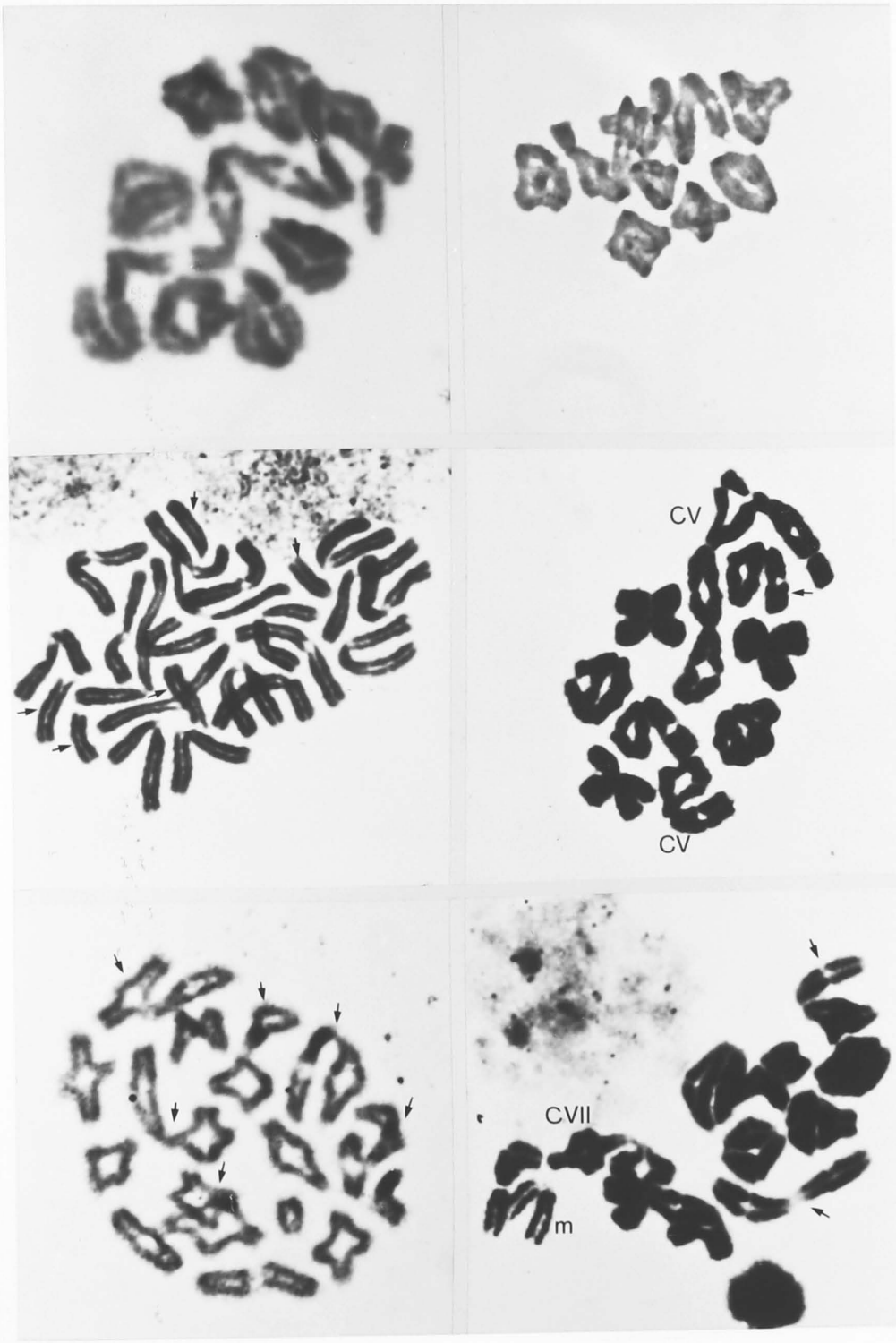

CV
CV
CVII
m

Figure 4.67 *D. cancerides*, male meiosis, hybrid derivative, diakinesis. This figure shows two of the chains of three chromosomes and one telocentric bivalent from the individual shown in Figure 4.65. Canberra ACT.

Figure 4.68 *Isopoda villosa*, male meiosis, pachytene. Synaptonemal complexes. Note the parallel alignment of the complexes. Canberra ACT.

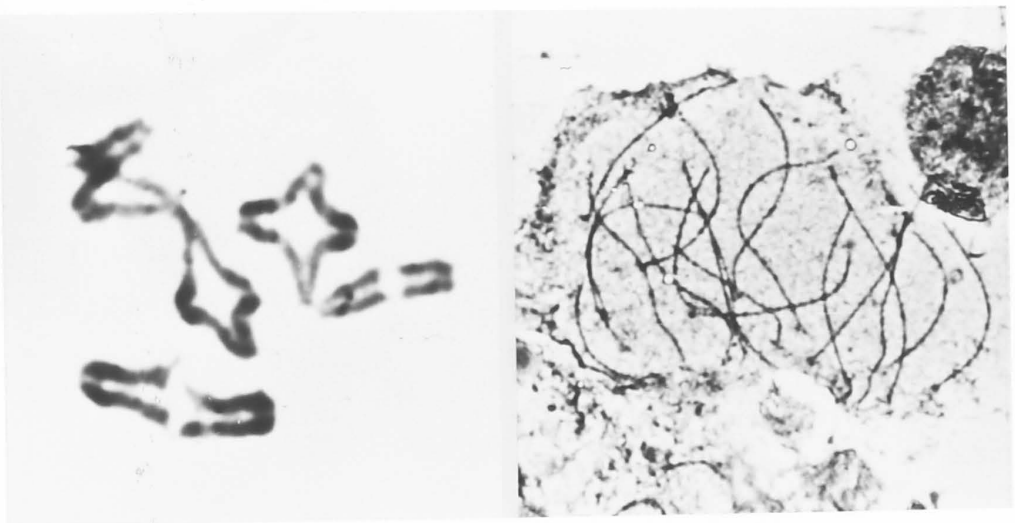


Figure 4.69 *Isopoda villosa*, male meiosis, synaptonemal complexes, late zygotene/early pachytene. 20 bivalents are visible in this preparation, two of which (arrows) have not fully synapsed. The darker, thickened regions are associated with the centromeres (this can be ascertained from later preparations of metacentric chromosomes, especially from Figure 4.77). Thus it can be seen that the non-synapsed regions in this figure are at the distal ends of the chromosomes. No structures associated with the X-chromosomes are evident in this figure. Canberra ACT.

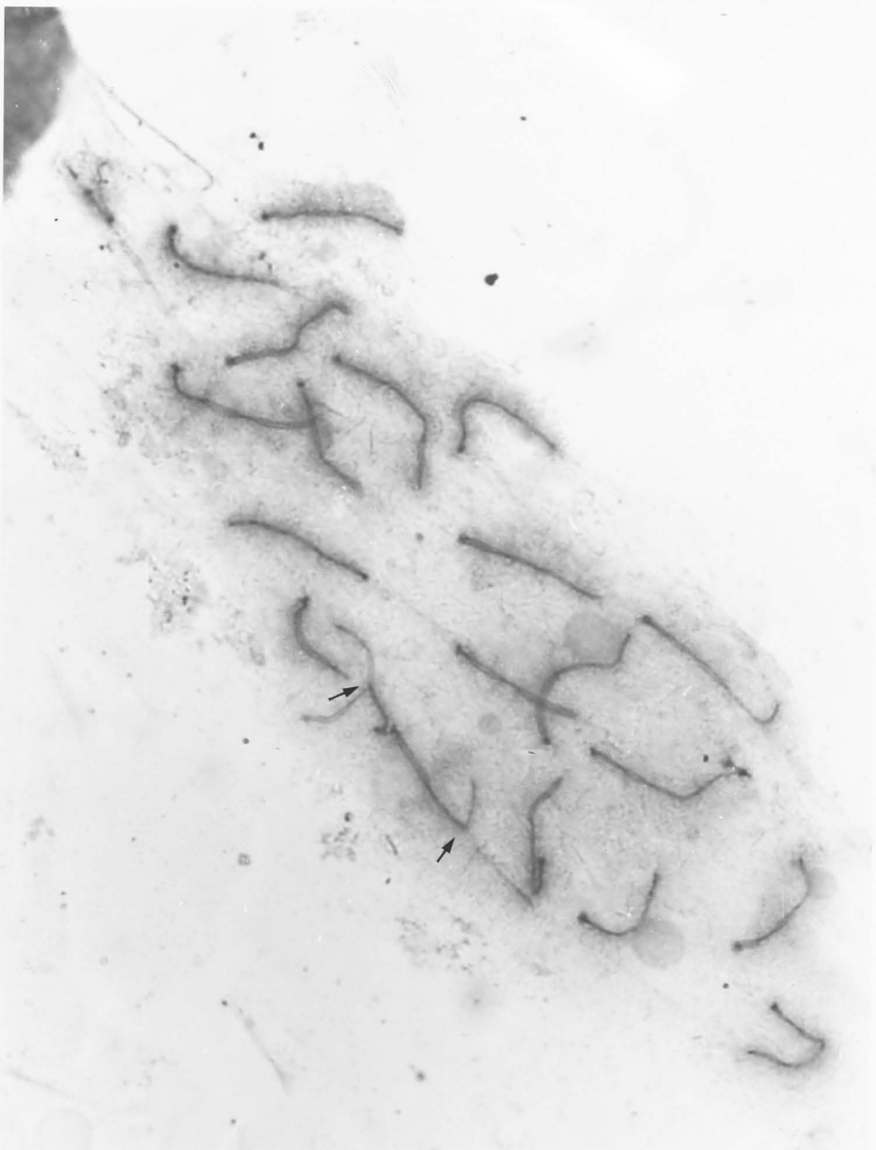


Figure 4.70 *Isopoda villosa*, male meiosis, synaptonemal complexes, pachytene. 20 fully paired bivalents + 3 X-associated elements (arrow). When these elements are visible in this species, the triradiate structure occurs consistently. These elements clearly differ from the lateral elements of the autosomes both in length and staining intensity. Canberra ACT.

Figure 4.71 *D. cancerides*, male meiosis, synaptonemal complexes, leptotene, tII race. Pairing has not commenced in this cell. Note the clustering of the centromeres, which mirrors the clustering seen in Figures 4.19 and 4.20. This suggests that pairing may commence proximally to the centromere where all of the chromosomes are in close proximity. Canne River Vic.

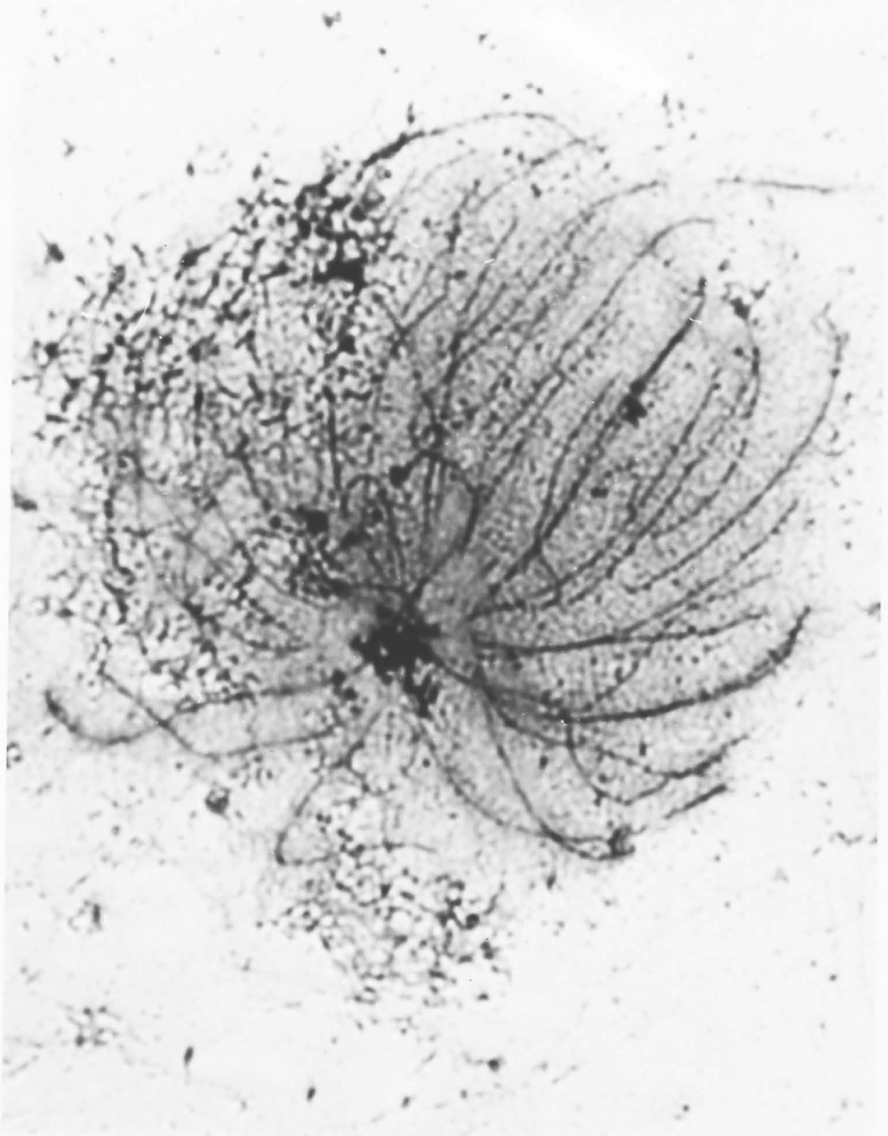


Figure 4.72 *D. cancerides*, male meiosis, synaptonemal complexes, pachytene, tII race. 20 fully paired bivalents. The dark structures associated with the centromeres are fully visible in this cell. No elements associated with the X-chromosomes are visible. Canne River Vic.

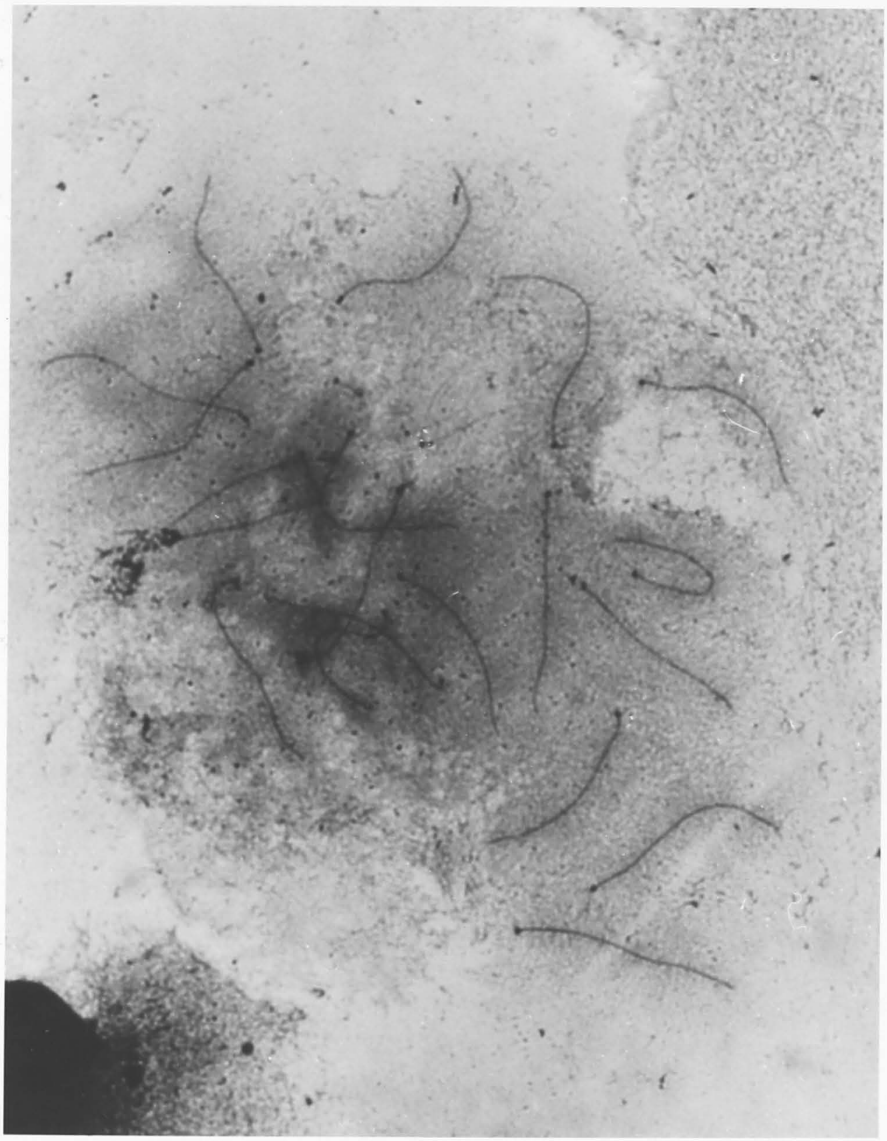


Figure 4.73 *D. cancerides*, male meiosis, synaptonemal complexes, pachytene, tII race. 20 fully paired X-chromosomes + 3 X-associated elements (arrow). The triradiate pattern formed by these elements is clearly visible. Canne River Vic.

Figure 4.74 *D. cancerides*, male meiosis, synaptonemal complexes, pachytene, CV race. 8 fully paired metacentric bivalents (■) + star-shaped structure (arrow) formed by the pairing of the chain chromosomes and association of the metacentric X-chromosomes. This structure is magnified in Figure 4.75. Glencoe NSW.

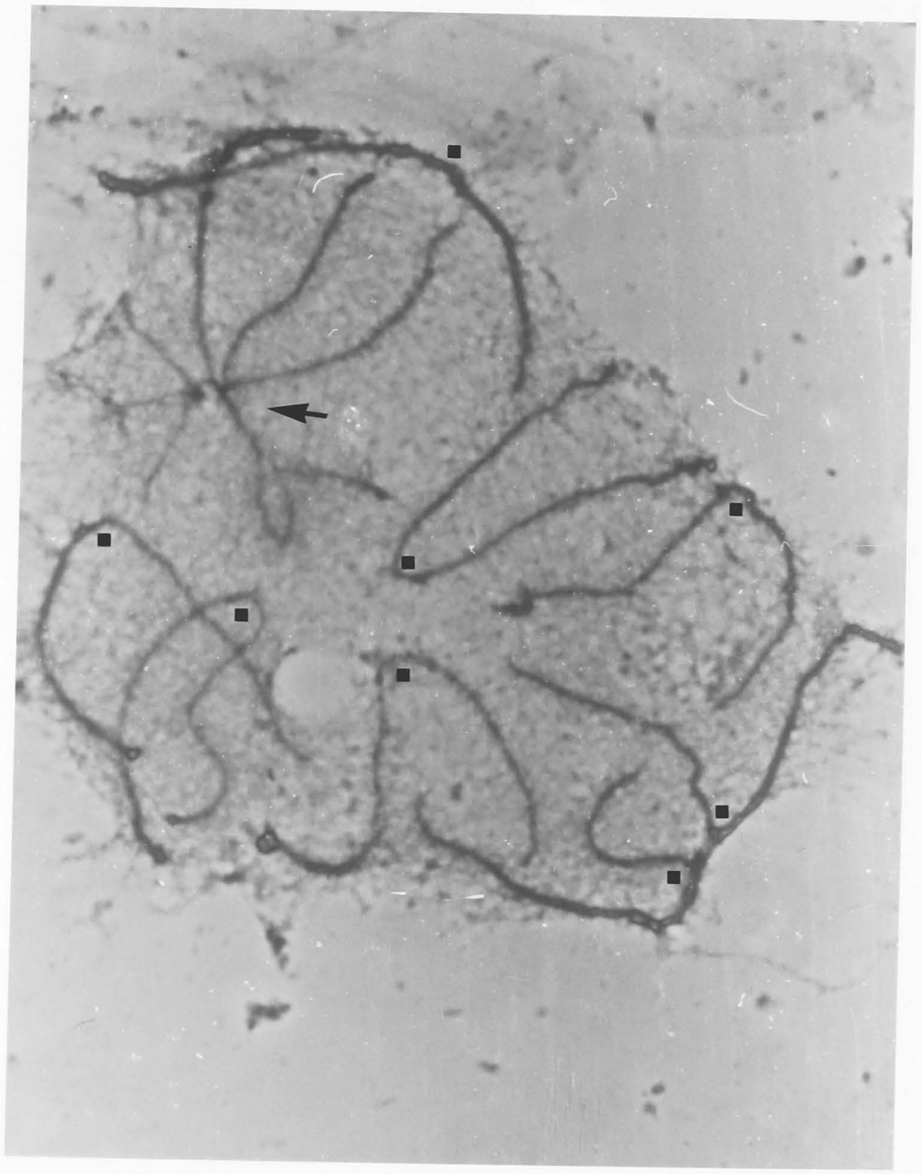
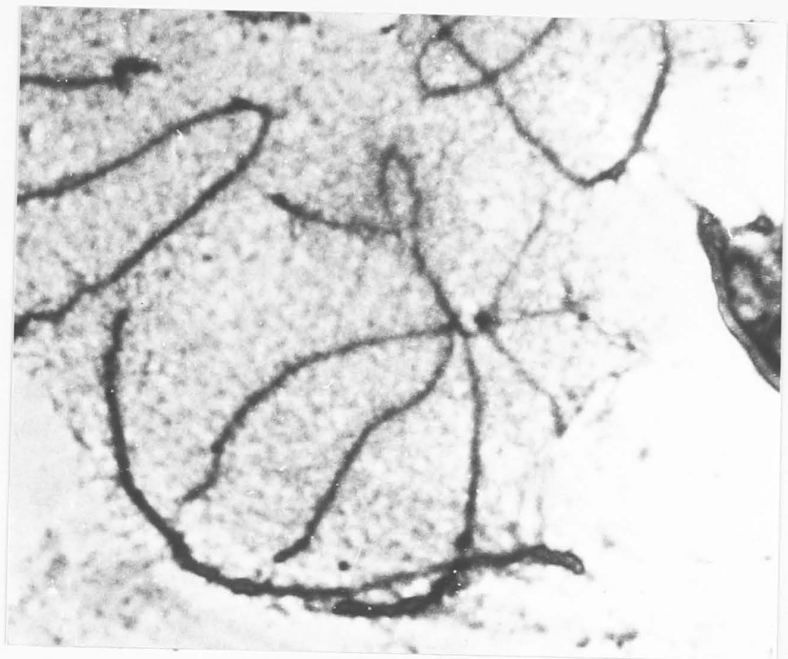


Figure 4.75 This figure shows a magnification of the structure shown in Figure 4.74, which is formed by the pairing of the chromosomes in the chain-of-five. A schematic representation of the mechanism by which this is formed is shown below. The four darker rays consist of the fully paired arms of the autosomes, while the three paler rays are the result of the association of the metacentric X-chromosome with the centromeric region of the X-A fusion product. This X-association is reminiscent of the triradiate structure formed by the X-chromosomes in the telocentric races and species (Figs 4.70, 4.73).



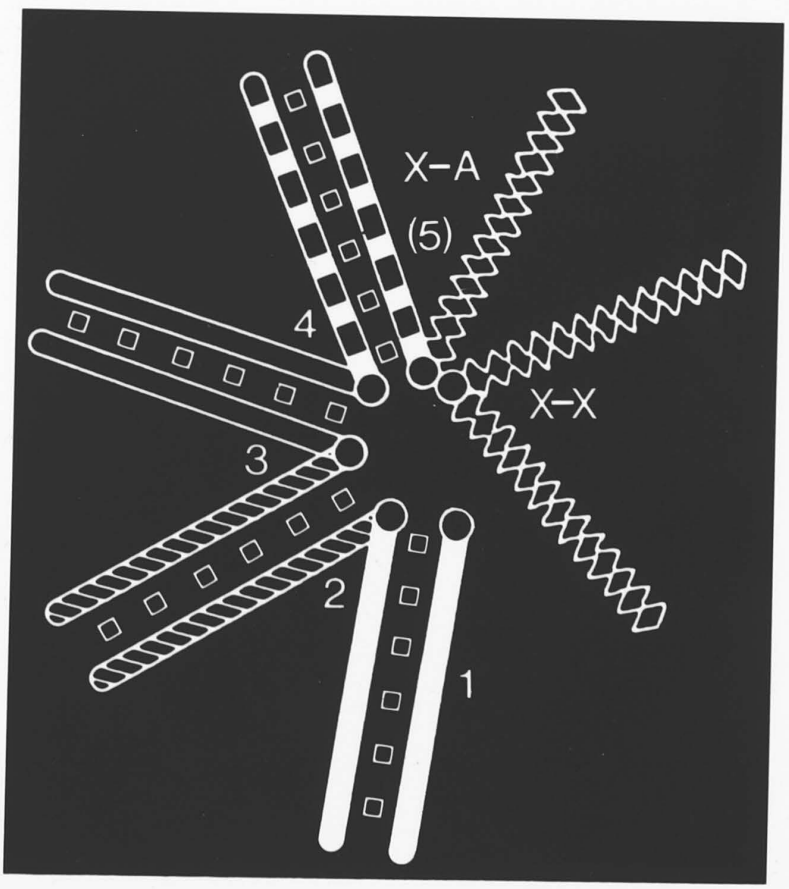

X–A
(5)
4
X–X
3
2
1

Figure 4.76 *D. cancerides*, male meiosis, synaptonemal complexes, pachytene, CIX race. Here, a structure consisting of eight dark rays and three shorter, paler rays is visible (arrow). Four of the darker rays are slightly separated from the rest of the structure, possibly as the result of breakage during preparation. Again, as in the CV race (Figs 4.74,4.75), the metacentric X-chromosome is associated with the structure. Wonboyn NSW.

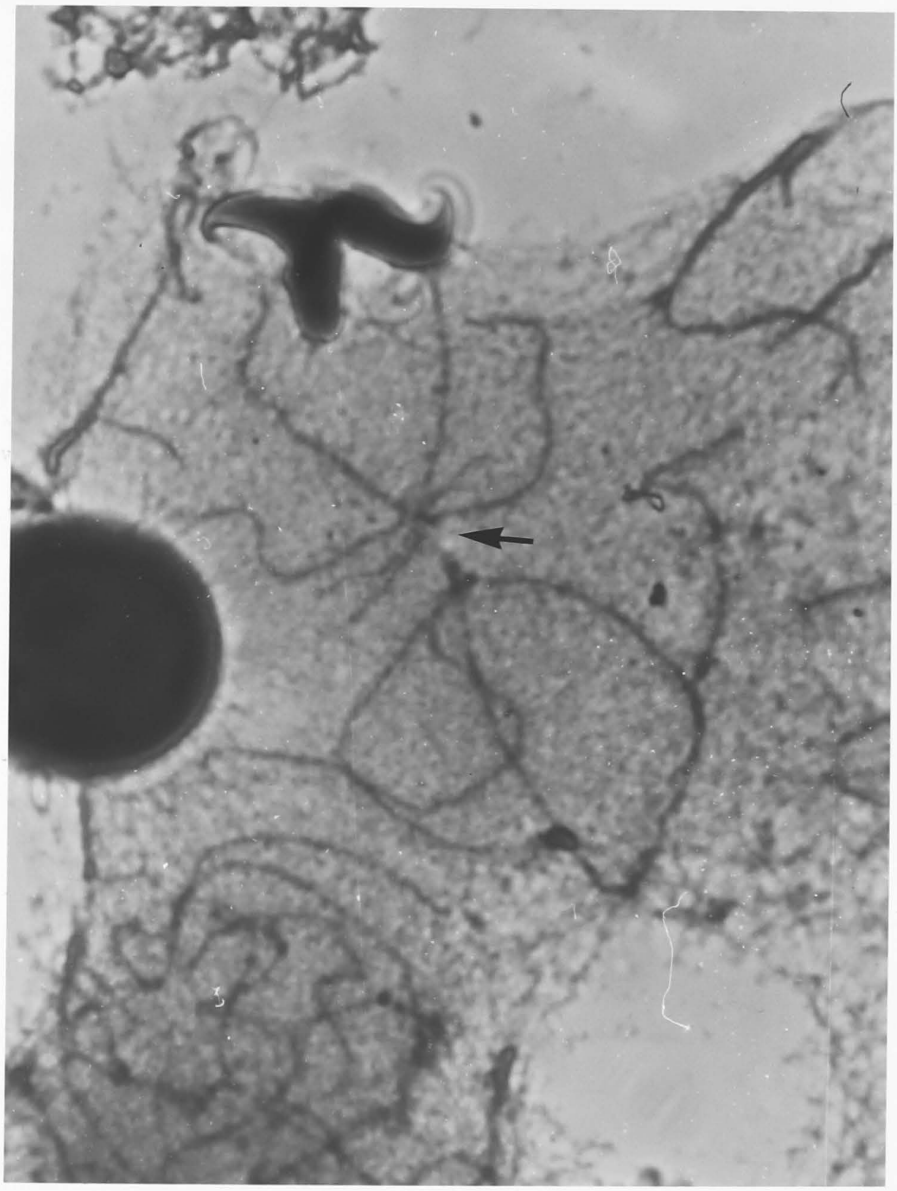


Figure 4.77 *D. cancerides*, male meiosis, synaptonemal complexes, zygotene, CIX race. The 6 metacentric bivalents have only partially synapsed, and pairing in these appears to be proceeding from the centromeric region towards the telomeres. In four of the bivalents the centromeric region is thickened and composed of two distinct positively staining bodies. The open arrows indicate fragments of the complexes associated with the chain-forming chromosomes. The original conformation of this structure cannot be reconstructed with any certainty, however it does appear that, in this fusion multiple, pairing has commenced in the interstitial regions of the chromosomes. This is particularly apparent in the region directly in front of the uppermost open arrow. This unpaired region is clearly located between two of the eight rays which will form the bouquet configuration at pachytene (Fig. 4.76). Thus pairing on at least one of these rays must be proceeding from an interstitial region towards the centromere. This also appears to be the case in the fragment to the lower right of this. Wonboyn NSW.

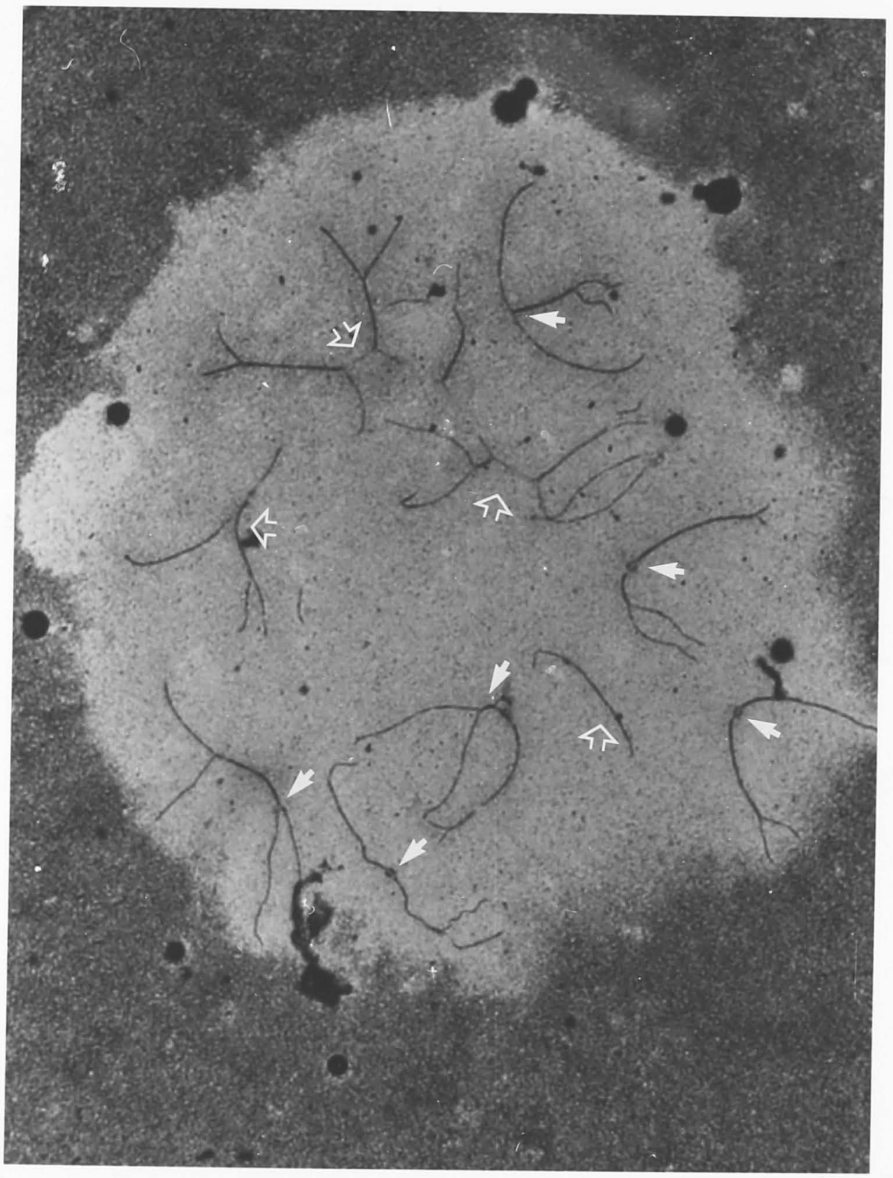


Figure 4.78 Collection sites for *D. cancerides* specimens karyotyped. Reference points within 20km of each site are listed below. The three individuals from the CIII race (Perth, WA) are not included.

NO.	RACE	NO. OF INDIVIDUALS	LOCALITY
1	tII	3	Cherwell Ck. 20km South of Gin Gin, Qld.
2	tII	1	15km South of Biggendon, Qld.
3	tII	1	Neara Ck. 20km West of Kilcoy, Qld.
4	CV	3	20km North of Glenn Innes, NSW.
5	CV	3	Glencoe, NSW.
6	CV	2	Armidale, NSW.
7	CV	2	Gunnedah, NSW.
8	CV	7	Tamworth, NSW.
9	CV	2	Raymond Terrace, NSW.
10	CV	1	Newcastle, NSW.
11	CV	1	Toukley, NSW.
12	CIX	2	Kuringai National Park, NSW.
13	CIX	2	Hall, ACT.
14	CIX	2	Wee Jasper, NSW.
15	CIX	1	Forbes Ck., NSW.
16	CIX	3	Araluen, NSW.
17	CIX	25	Coastal, Jervis Bay to Wonboyn, NSW.
18	CIX	2	Nimmitabel, NSW.
19	tII	1	Canne Valley Highway, Vic.
20	tII	1	Canne River, Vic.
21	tII	2	Bairnsdale, Vic.
22	tII	1	Aboriginal Museum, Lakes Entrance, Vic.
23	mII	4	Benalla, Vic.
24	mII	1	Seymour, Vic.
25	mII	1	Castlemaine, Vic.
26	mII	3	Dunneworthy, Vic.
27	mII	2	Glenthompson, Vic.
28	mII	1	Horsham, Vic.
29	mII	1	Hall's Gap, Vic.
30	mII	1	Millicent, SA.
31	mII	1	Greenways, SA.
32	CIX	2	Bugaboi, Vic.
33	CIX	1	Hattah Lakes, Vic.
34	mII	2	Kangaroo Is. SA.
35	CIX	3	Adelaide area, SA.
35	mII	2	" " "
36	tII	2	Evandale, Tas.

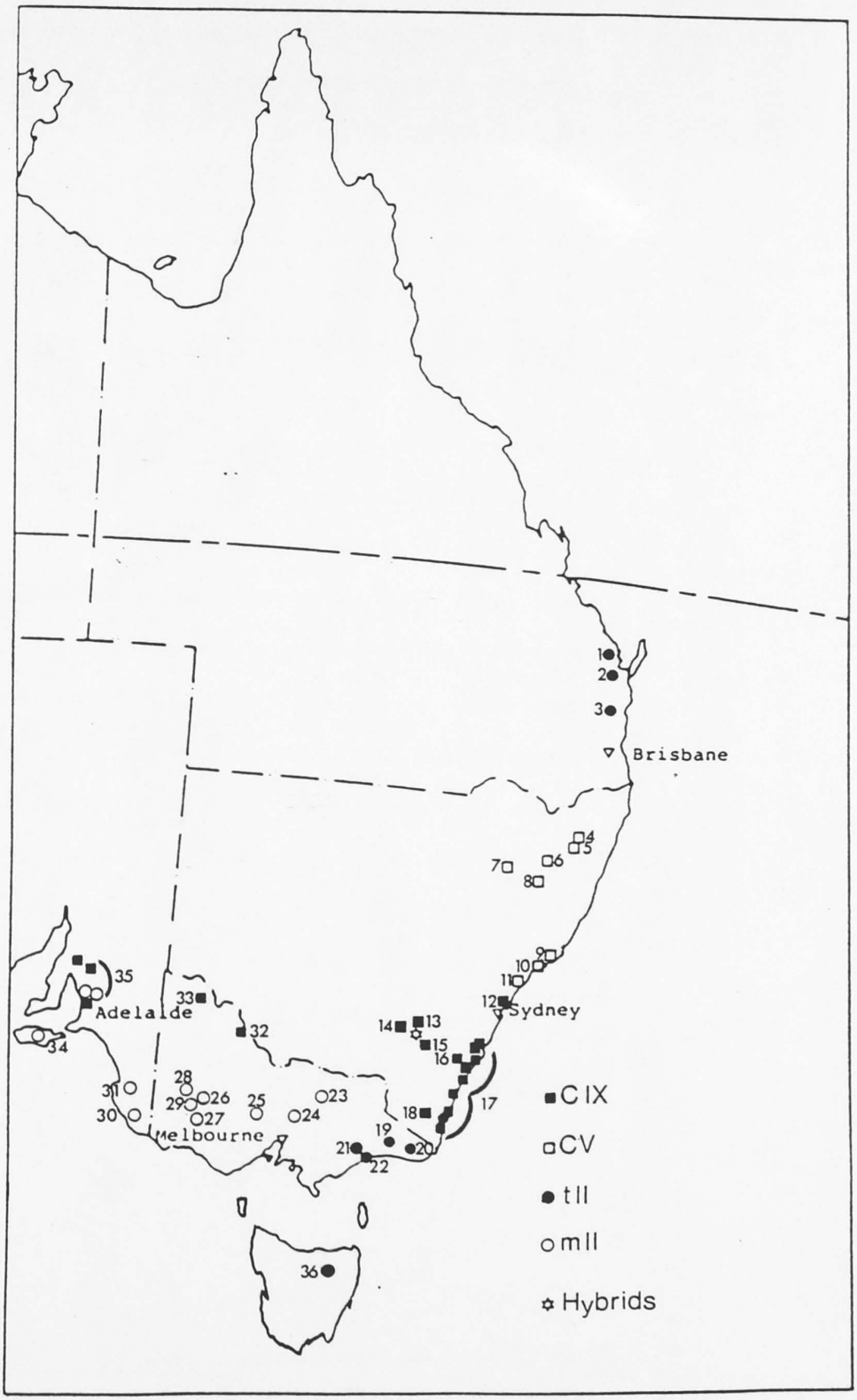

Brisbane
Adelaide
Melbourne
Sydney
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
C IX
CV
t II
m II
Hybrids

Figure 4.79 Schematic representation of the chromosome complement and meiotic configurations of males and females of the CIX race of *D. cancerides*. Chromosomes 1,3,5,7 and 9 ("A" haplotype) are homozygous in females, while males are heterozygous for the two chromosome groups - the X-linked A haplotype (1,3,5,7 and 9), and the B haplotype (2,4,6 and 8) which is not associated with an X-chromosome. All gametes from females possess the A haplotype. The B haplotype of the males is passed on to male offspring only, since it segregates from the X-chromosome, and so male offspring are heterozygous for the two complexes. The A haplotype in males gives rise to female-determining sperm and combines with the A haplotype in the female gametes. Consequently female offspring are always homozygous for the A haplotype, and so presumably form bivalents at meiosis, and males are perpetually heterozygous for the two groups, and form chains. Thus, this system behaves as an $X_{1-5}Y_{1-4}$ sex determining system, with the chromosomes of the A haplotype analogous to X-chromosomes and those of the B haplotype behaving as Y-chromosomes.

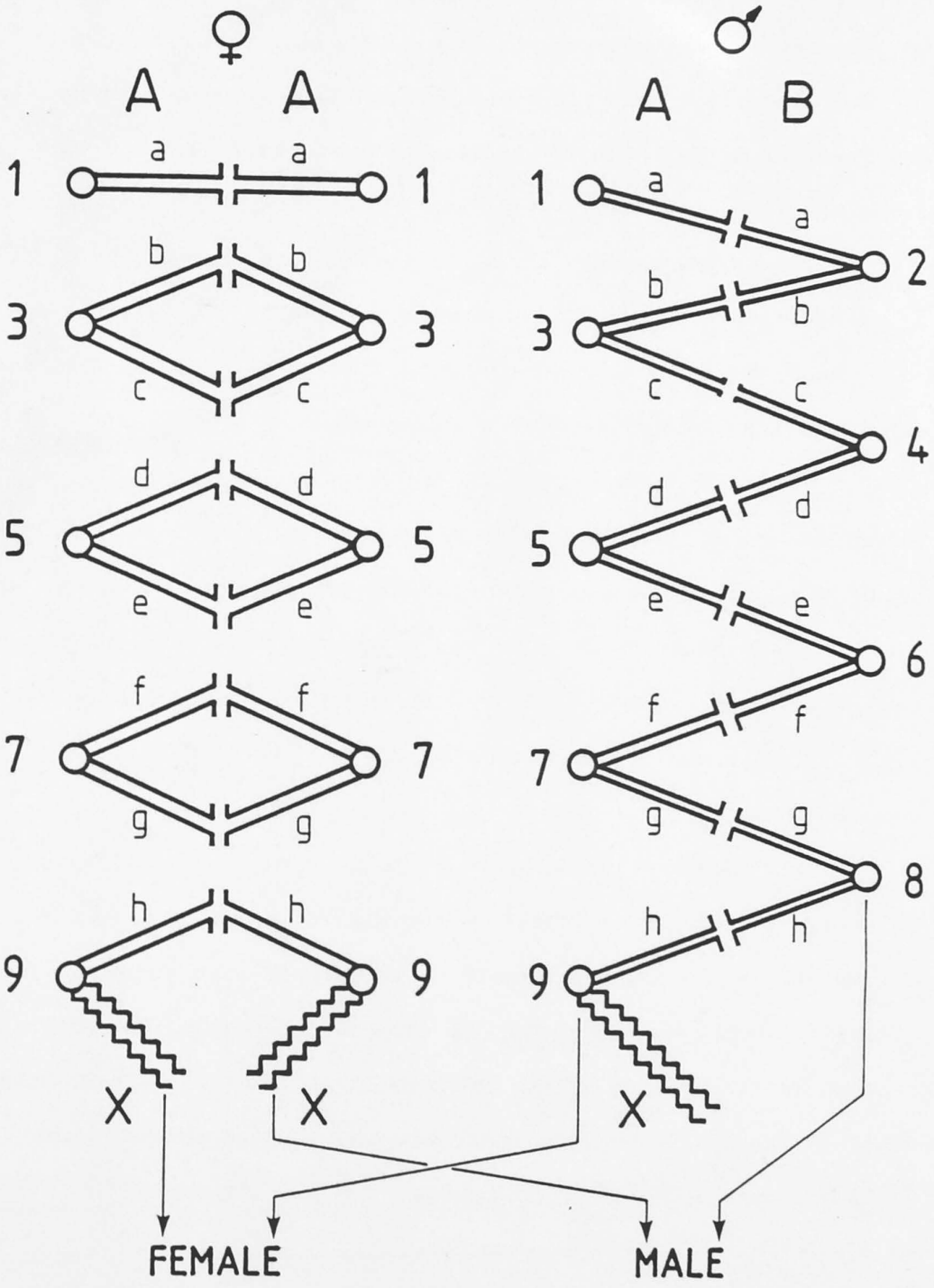
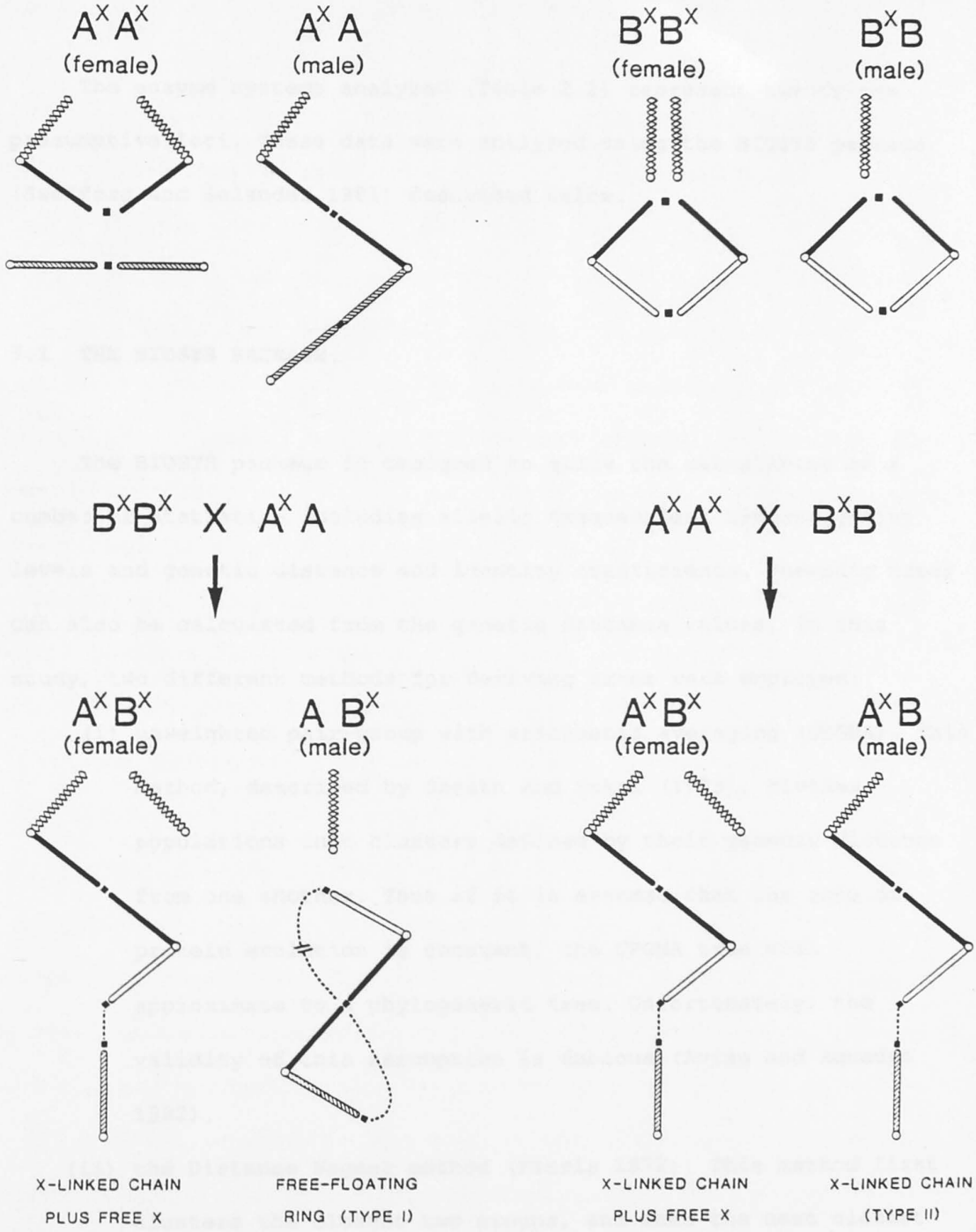

♀
A A
♂
A B
1
a a
1
1
a a
2
3
b b
3
3
b b
c c
c c
4
5
d d
5
5
d d
e e
e e
6
7
f f
7
7
f f
g g
g g
8
9
h h
9
9
h h
X
X
X
FEMALE
MALE

Figure 4.80 Schematic representation of the products of hybridisation between two chromosome races, AA and BB. These two races have complete homology between chromosome arms, but carry different fusion combinations. Both possess 21 metacentric chromosomes and one telocentric in the male and a second telocentric in the female, but only the relevant chromosomes are shown here. In the AA race the telocentric chromosome is an autosome and consequently males of this race form a chain-of-three at meiosis. The BB race is homozygous for all of the fusions, forming bivalents at meiosis; the telocentric chromosome is an X.

Both reciprocal crosses result in females carrying an X-linked chain and the free telocentric X-chromosome.

Since the X-chromosome in the BB race is unfused, the male offspring of the BB female by AA male cross carry a fusion multiple which is unassociated with the telocentric X-chromosome. This multiple is a ring, since it is not terminated by a telocentric autosome or the X-chromosome. This is termed a "type I" male, and since the multiple is not sex-linked it will not be maintained in future generations.

The AA female by BB male cross yields type II male offspring which carry a sex-linked chain of the kind observed in the CV and CIX races. If these males were to backcross with the AA females, the offspring would be karyotypically identical to their like-sex parent, as is the case in the chain carrying races of *D. cancerides*. Thus, if the chain carrying races do have a hybrid origin, some mechanism must exist which selectively removes the female and type I male hybrids.


A^XA^X
(female)
A^XA
(male)
B^XB^X
(female)
B^XB
(male)
B^XB^X X A^XA
A^XA^X X B^XB
A^XB^X
(female)
$A\,B^X$
(male)
A^XB^X
(female)
A^XB
(male)
X-LINKED CHAIN
PLUS FREE X
FREE-FLOATING
RING (TYPE I)
X-LINKED CHAIN
PLUS FREE X
X-LINKED CHAIN
(TYPE II)

CHAPTER 5.

PROTEIN ELECTROPHORESIS

The enzyme systems analysed (Table 2.2) represent twenty-one presumptive loci. These data were analysed using the BIOSYS package (Swofford and Selander 1981) described below.

5.1 THE BIOSYS PACKAGE.

The BIOSYS package is designed to allow the calculation of a number of statistics including allelic frequencies, heterozygosity levels and genetic distance and identity coefficients. Phenetic trees can also be calculated from the genetic distance values. In this study, two different methods for deriving trees were employed:

(i) unweighted pair-group with arithmetic averaging (UPGMA). This method, described by Sneath and Sokal (1973), divides populations into clusters defined by their genetic distance from one another. Thus if it is assumed that the rate of protein evolution is constant, the UPGMA tree will approximate to a phylogenetic tree. Unfortunately, the validity of this assumption is dubious (Avise and Aquadro 1982).

(ii) the Distance Wagner method (Farris 1972). This method first clusters the closest two groups, and then the next closest group to the nodal point (hypothetical common ancestor) of the initial two groups. Subsequent groups are included on the basis of their distance from the new nodal point, which will of course change with the inclusion of each new group. The

Distance Wagner method does not assume a constant rate of protein evolution. The root point of the tree can be defined as the midpoint of the longest arm, or as a previously defined outgroup. If the outgroup is separated from the "ingroup" by a large genetic distance, the outcome will be the same, whichever method of rooting is chosen.

UPGMA trees were derived using the genetic distance coefficients of Rogers (1972) and Nei (1978). The distance coefficient of Nei (1978) is based on the earlier genetic distance measure of Nei (1972), but while the earlier measure neglects the effects of polymorphism, the later version does not. It is claimed that Nei's genetic distance coefficient is superior to other distance coefficients since other measures are "constructed from the statistical point of view", while Nei's distance "is intended to estimate the number of net codon differences per locus between populations" (Nei and Roychoudhury 1974), and consequently has more biological significance. No data have been published to test whether Nei's intention has been realised, but it has been observed that the various different measures of genetic distance that are used in electrophoretic studies are highly correlated (Gorman and Renzi 1979). For Wagner trees only the Rogers' distance estimates are used since, for statistical reasons, the Nei coefficient is inappropriate (Baverstock *et al.* 1982).

When the distance between taxa is small, both Nei's and Rogers' coefficients are prone to large standard errors, and the major factor influencing standard error is the number of loci used rather than the sample size (Nei and Roychoudhury 1974, Nei 1978, Gorman and Renzi 1979, Baverstock *et al.* 1982). "Small" Nei distances are generally held to be distances of less than 0.1.

Two measures of "goodness of fit" are given for the trees derived in this study - the cophenetic correlation and percent standard deviation. The cophenetic correlation is a measure of the correlation between the distances calculated from the raw data and the distances derived from the tree. A correlation of 1.0 indicates that the tree perfectly mirrors the distances calculated, while a value of 0.0 indicates that extreme distortion of the observed values has been necessary to derive the tree. The percent standard deviation (Fitch and Margoliash 1967) is a function of the sum of the differences between the calculated distance for each pairwise comparison and the distances between the groups read from the derived tree. Individual values for this variable have no real meaning *per se*, but are valuable for estimating the comparative "correctness" of trees derived using different methods or distance coefficients.

A problem with genetic distance coefficients, on which both of these methods rely, is that they do not distinguish between shared ancestral (symplesiomorphic) and shared derived (synapormorphic) character states (terminology of Hennig 1965, 1966). Thus if two groups are phylogenetically distant but share many plesiomorphic (ancestral) character states through their common ancestor, the genetic distance between them will be small. Hence, while the Wagner method may give a better approximation of the branching sequence of a phylogeny (Baverstock *et al.* 1982), the results from any method of clustering which does not distinguish between plesiomorphic and apomorphic (derived) character states should be treated with caution. Unfortunately, phylogenetic analyses based on Hennigian principles (Hennig 1965, 1966) can only be carried out when discrete character states are identifiable and their evolutionary status (plesiomorphic or apomorphic) can be ascertained.

5.2 COLONY STRUCTURE

Cohorts of juveniles from colonies were identified as groups of individuals of similar size, with a distinct discontinuity between these and the next highest and lowest size classes. One cohort along with the single adult female present in each colony was analysed electrophoretically in order to determine whether, in each case, the offspring were the result of a single mating. In the colony from Tamworth an adult male was also collected and included in the sample. The results are shown in Table 5.1. Only variable loci are shown. The colonies were analysed separately, and so the allelic designations in Table 5.1 do not correspond between the colonies.

It was initially assumed that the adult female in each colony was the parent, but this is unlikely because in all three of the samples it is impossible to derive all of the juvenile karyotypes from that of the adult female, irrespective of whether or not multiple matings are assumed. Furthermore, the adult male from the Tamworth colony cannot be the common parent of all of the juveniles. These conclusions were drawn from the following observations:

(i) In colony 1, juvenile 6 is homozygous for the A allele of aldolase, while the adult female is homozygous for allele B. Similarly, for juvenile 7, the AB genotype for MPI cannot be derived from the CC female.

(ii) In colony 2, the four juveniles homozygous for the MPI A allele could not have had the BB female as their parent.

(iii) In colony 3, the genotypes for PGM (juvenile 3) and IDH-1 (juvenile 6) must have been received from parents carrying the D and C alleles respectively.

In colonies 2 and 3 the adult females could be parents of the juveniles if, at the loci in question, they were heterozygous for a

null allele. Thus they would appear to be homozygous for the single active allele they possessed, but this allele would not always be present in the offspring. Offspring that received the null allele would then appear to be homozygous for whatever active allele they received from the male parent. This cannot explain the MPI genotype of juvenile 7 in colony 1 however, since it possesses two alleles, neither of which is present in the female.

5.3 COMPARISONS OF *D. CANCERIDES* RACES

5.3.1 Samples.

Samples from all of the chromosomal races were analysed electrophoretically. These samples are shown in Table 5.2. With the exception of the Kangaroo Island sample, each individual was collected from a separate tree to avoid any bias resulting from the inclusion of siblings. Even so, owing to the absence of any data on dispersal patterns in this species, it is possible that individuals collected from nearby trees were related.

The Kangaroo Island sample was not collected by me, and unfortunately no collection details are available. For this reason, and that there is unlikely to have been recent gene flow between this population and the mainland, the data for this sample were not pooled with the mainland data for the mII race. Similarly, the three tII populations from Queensland, Victoria and Tasmania are clearly genetically isolated. Owing to differences in allelic frequencies detected between the South Australian and Victorian mII samples, these were also kept separate during data analysis. As was discussed in chapters 1 and 2, complex sex-linked fusion heterozygosity can potentially restrict some alleles to males only, and so to determine

whether this has indeed occurred, males and females from the CV and CIX races were initially analysed separately.

The raw electrophoretic data are shown in Appendix 1.

5.3.2 Loci used.

With the exception of MPI, all of the loci given in Table 2.2 were stained for these samples. In MPI, at least twenty-two alleles were identified and the mobilities of many of these were so similar that they could only be distinguished from one another when loaded adjacently on the gels. Insufficient time and resources were available to attempt every necessary combination of samples and furthermore, given the extreme variability of this system, it is unlikely that it would contribute any useful information.

5.3.3 Gene Frequencies.

The allelic frequencies for each population are shown in Table 5.3. Only one individual from Tasmania was included, and so the frequency data for this sample have little meaning. Among the other populations the commonest allele differed at only three loci.

- at the IDH1 locus in the CV and CIX populations allele B was the most frequent, while in the other populations, allele C was more common. Allele C was also relatively common in these two chain-carrying populations however, at a frequency of between 30% and 50%.

- allele B of the AAT1 locus was the most frequent in the northern tII sample, but allele E was consistently the most frequent allele in all of the other populations. Allele B was also detected at low frequency in the Victorian tII, CIX and CV races.

- there was a fixed difference between the Queensland and Victorian tII samples for aldolase, while in the two larger mII

samples and the CIX and CV samples both alleles were detected. The single tII individual from Tasmania shared the B allele with the Queensland tII sample, and in the CIII sample allele C was absent. Allele B was not detected in the Kangaroo Island mII sample but results were obtained for only four individuals, and these may have been closely related.

Differences in gene frequencies between the Victorian and South Australian mII samples are evident at the PGD, MDH1, GPI, PGM, IDH1, AAT1 and ALD loci.

It is of interest to note that the aldolase B allele occurs in both Queensland and Tasmania but was not detected in the geographically intermediate Victorian tII sample. Since this allele occurs in both the Queensland and Tasmanian tII samples, and the tII condition is clearly ancestral to the fusion races, it is probable that this allele represents the plesiomorphic state. Similarly, the fact that the AAT2 A allele occurs only in the Queensland tII, CIX and Tasmanian tII samples may indicate that it is also ancestral; alternatively, it may occur in the other populations but has not been detected.

Three males from the CIX sample were heterozygous for allele C at the aldolase locus and a null allele of similar mobility to allele B. Since aldolase is a tetramer these genotypes were seen as four bands on the gel, the bottom two of equal intensity and the next two progressively paler. None had sufficient contrast to photograph successfully. In these three cases, the individuals were scored as BC heterozygotes since their low frequency would have a negligible effect on genetic distance estimates, and in the analysis of sex-linkage below, their main significance is that they are "not C".

5.3.4 Sex-Linkage of Alleles.

In both the CV and CIX samples, relatively large frequency differences were observed between the two sexes for alleles B and C of the aldolase locus. Using the *G*-test (Sokal and Rohlf 1981), this difference was found to be statistically significant for the CIX race but not for the CV race (Table 5.4).

Table 5.4 also shows the genotype frequencies for the CIX population and from these it is possible to estimate the frequency of the two alleles on the X- and Y-complexes as follows. Because both alleles are present in females, which possess only the X-complex, both must be present on the X-complex complex and the calculation of the frequencies is straightforward - 14.5% and 85.5% respectively. In males, which have both the X- and Y-complexes, no CC individuals are present. Given the high frequency of the C allele on the X-complex, the absence of CC males, combined with the large number of male BC heterozygotes, indicates that this allele is rare or absent on the Y-complex. Assuming the C allele to be completely absent on the Y-complex, the frequency of the alleles on the X-complex is calculated to be 19.6% and 80.4% respectively. This assumption can be tested by comparing the predicted frequency of the alleles on the X-complex estimated from males with the estimate from females, as shown in Table 5.4. The absence of any significant difference between the two pairs of estimates indicates that this assumption is reasonable.

The aldolase gene is most probably on the homologous arms of the third and fourth chromosomes of the chain-of-nine, in which crossing over always occurs distally, since they possess the bulk of the genetic material that is consistently sex-linked. Alternatively this gene may be present on one of the other chromosomes of the chain in a position more proximal than the most proximal chiasma.

None of the other enzyme loci show as large frequency differences between the sexes as does aldolase in the CV race in which it is not statistically significant, and so aldolase is clearly the only sex-linked locus and it is sex-linked only in the CIX race.

5.3.5 Aldolase staining.

An AB heterozygote for a tetramer has five forms, AAAA, AAAB, AABB, ABBB and BBBB. These forms occur in the in the ratio 1 : 4 : 6 : 4 : 1 if both alleles are transcribed and translated at the same rate and association is random. Thus when electrophoresis is carried out, five bands will be visible with staining intensities in this ratio. In aldolase BC heterozygotes, however, this was not always the case. In some instances the middle and next highest band were of equal intensity and darker than the others (Figures 5.1 and 5.2) or else the second top band was the most intense. All heterozygotes stained with the same pattern on any one gel, but the same sample run on three separate occasions sometimes showed all three of these patterns. Particular staining patterns were found to be completely independent of the age of the sample, the grinding buffer and the running buffer. Assuming that the tetramers do not dissociate and non-randomly recombine in the samples, the most likely explanation for this is that under certain conditions the second top band (BBBC) has more activity.

Since aldolase is clinally distributed on the eastern Australian mainland, with the B allele fixed in the north and the C allele fixed in the south, the possibility that there is an adaptive reason for this, related to temperature, was considered. That is, if the B allele functions more efficiently at higher temperatures, it would be selected for in the north, while the C allele would be adaptively superior in the cooler more southerly regions. Thus in the heterozygotes, staining at high ambient temperatures would result in

greater intensity of the more B-rich tetramers. To test this, two gels were run at the same time, using the same running buffer and samples, one at 4°C and one at 26°C, and stained at these temperatures. Both gels showed the same staining pattern, with the middle and next highest band of equal intensity.

5.3.6 Canberra Hybrids.

In the last chapter, it was pointed out that the most likely origin for the mixed population collected from Canberra involved hybridisation between the CIX race and either the Victorian or Queensland tII race. If the tII parent race were from Queensland, the AAT1 B allele which is predominant in this race would be expected to occur at a higher frequency in the hybrid population than in the CIX race. If on the other hand the Victorian population is the tII parent, the E allele predominant in this race should be more common in the hybrids. That the latter is the case supports the argument that the Canberra population has resulted from hybridisation between the CIX race and the Victorian tII population. Similarly, the predominant aldolase allele of the Victorian tII population is at a higher frequency in the hybrids than in the CIX sample, which further supports a CIX x Victorian tII origin.

The sex-linkage of aldolase is still present in the hybrid sample which suggests that either the hybridisation events proposed are recent, or that the CIX karyotype is more common than the tII karyotype in this area.

5.3.7 Allele number.

If the hybridisation model for the origin of the chain races (section 4.4.5(a)) is correct, then the chain races may be expected to possess more alleles than either parental race, since they could have

gained alleles from both. Unfortunately the samples from the different races cannot be directly compared because they are different sizes and more of the rarer alleles will have been detected in the larger samples. It is possible to devise a statistical test for comparing the allele number in the different samples however, if it is assumed that the CV and CIX samples are sufficiently large to be an accurate representation of the true number of alleles in these two races, and that the gene distributions within these two races are homogeneous throughout the ranges sampled. The former is considered to be a reasonable assumption for the following reasons:

The probability of *not* detecting an allele of frequency p in a sample of size n is $(1-p)^{2n}$; '$2n$' because the animals are diploid so from each individual examined there are two chances of detecting a given allele. Thus the minimum possible frequency of an allele at which there will be a 95% chance of detection can be calculated from the following expression: $(1-p)^{2n} = 0.05$. For the CIX (n=92) and CV (n=50) samples $p = 0.016$ and 0.030 respectively. That is, 95% of the time alleles with a frequency of 1.6% and 3% respectively will be detected. Using the same formula, 50% of the time alleles with a frequency of 0.4% and 0.7% will be detected in the CIX and CV races.

95% confidence intervals for an observed frequency p′ can be calculated using the formula $p' \pm (\sqrt{p'(1-p')/2n}) \times 1.96$. 95% confidence intervals for observed frequencies of 10%, 50% and 90% are shown below for the CIX and CV samples.

p′	CIX (n=92)	CV (n=50)
10%	± 4.3%	± 5.9%
50%	± 7.2%	± 9.8%
90%	± 4.3%	± 5.9%

Thus the sample sizes from these two populations provide a reasonable approximation to the true allelic distributions.

The second assumption is difficult to test because of the small sizes of the subsamples used for these two races (Table 5.2). However, since the frequencies of the commonest alleles are similar even in groups as widely separated geographically as the CIII and Victorian tII populations, this assumption is considered reasonable.

Given this, if a number of samples of fourteen individuals are taken, with replacement, from the CIX sample of ninety-two, the distribution of the number of alleles observed will approximate to the distribution that would be observed if samples of fourteen were taken from the true population. Thus if the number of alleles observed in the sample of fourteen individuals from the Victorian mII race is compared with the distribution generated from the CIX sample, the probability that the mII sample is derived from a population similar to the CIX race can be estimated. That is, given the null hypothesis that the allele number in the two races is the same, the probability that any one of the samples taken from the CIX race will have a larger number of alleles than the mII sample is 50%. Therefore, from the number of CIX samples of fourteen that have more alleles than the mII sample, the probability can be read from tables of cumulatively summed binomial probability. If the number of alleles in the single sample falls below the numbers from all of the ten subsamples of fourteen, then the probability of the null hypothesis being true is less than 0.001 and so it is rejected at the 5% level. If one of the ten samples falls below the single sample, as is the case for this example, the probability is less than 0.0107, and again the null hypothesis is rejected. If two of the ten are lower, the probability is 0.0547, and so the null hypothesis cannot be rejected.

The test described above was carried out for all of the samples, in each case using random samples of a size corresponding to that of the sample being tested, from both the CV and CIX races. In one case

(CV vs Queensland tII), one of the ten fell below, one was equal, and eight were above the single sample. In this case, a probability of 0.0327 (halfway between 0.0107 and 0.0547) was assumed. The CV and CIX races were compared using the Wilcoxon nonparametric ranking test (Walpole 1974). This was carried out twice, first using random samples of eight individuals and then twenty. In both cases the null hypothesis was rejected at the 1% level. The results of these tests are shown in Table 5.5. The aldolase results were not included in this analysis since the rules governing retention or loss of alleles are clearly different for sex-linked loci. The CIX race showed significantly more alleles than all of the other races including the CV race. In the CV race, significantly more alleles were detected than in the CIII, Queensland tII and Victorian mII samples.

5.3.8 Heterozygosity.

Mean heterozygosity levels per locus per individual and standard errors are given in Table 5.6. The highest levels are found in the CIX race where males have a level of 0.088 and females 0.104. This is not unexpected, since this race has significantly more alleles than the others (see above) and a larger mean number of alleles per locus (Table 5.6). Nevo (1978) has demonstrated that such a correlation between heterozygosity and number of alleles holds for most groups. While this difference in heterozygosity levels is not statistically significant for any pairwise comparison (Table 5.6), the fact that males and females of this race, which can be considered as independent samples, *both* have higher levels than the other races suggests that it is a real effect. The high level for the Canberra individuals (0.083) is doubtless a result of their hybrid origin resulting in an intermediate level of heterozygosity, between those of the CIX and Victorian tII parents. The heterozygosity value for the CV sample is

higher than those of the three samples which have a significantly lower number of alleles (see Table 5.5).

Males in the CV and CIX races did not show a significantly higher level of heterozygosity than females, which would be expected if any of the loci were sex-linked in the same manner as aldolase.

The heterozygosity levels are well within the range reported for other arthropods, of 0.0 - 0.309 (Nevo et al 1984), and other spiders (Table 1.4). The percentage of polymorphic loci is considerably higher than the values for other social spiders (Table 1.4), but is within the range observed for other arthropod species (Nevo *et al.* 1984).

Expected heterozygosity levels, assuming Hardy-Weinberg equilibrium, are also included in Table 5.6. The fact that the observed levels are generally lower than those predicted is attributable to the Wahlund effect, since the populations comprising the samples were often widely separated geographically and gene flow between them must be low or absent.

5.3.9 Genetic distances.

Rogers' (1972) distance and Nei's (1978) distance and similarity coefficients were calculated for pairwise combinations of populations (Table 5.7). Aldolase was not included because its inheritance in the CIX race does not result from random segregation.

In the CIX and CV races, it is possible that there is sex-linkage at other loci that was not evident from the allelic frequencies. If this is the case, the combination of a number of these could increase the genetic distance between the sexes. For this reason, the sexes were treated separately in these two races. No difference was detected between the sexes using the Nei distance and similarity coefficients and the Rogers' distances were small (Table 5.7), and consequently it is concluded that none of the other loci examined are consistently sex-linked.

The Rogers' distance coefficients were larger than the Nei coefficients, but followed the same general pattern.

The identity between the races was consistently greater than 0.94 except for the comparisons with the Tasmanian tII individual, which yielded similarity coefficients of around 0.8. Similarities of this magnitude have been reported between subspecies and sibling species in other invertebrate groups (Ayala 1975). The Victorian and South Australian mII samples which were thought to be distinct on the basis of the frequency data are, in fact, very similar, with distance and similarity coefficients of 0.001 and 0.999 respectively.

BIOSYS does not calculate errors for the distance measurements. In order to estimate the effect of sampling error, pairwise comparisons were made between the samples of twenty individuals from the CIX race which were used in the earlier analysis of allele number (Table 5.8). For the Nei distances, six of the forty-five comparisons yielded values of 0.003 or greater, and ten of the Rogers distance coefficients were 0.04 or greater. On the basis of this, it is concluded that the differences detected between the sexes in the CIX and CV races and between the Victorian and South Australian tII samples are the result of sampling error. Consequently, in the clustering analyses following, the sexes were combined in the CV and CIX races, and the two mII samples were treated as derivatives of the same population. Despite the fact that the Kangaroo Island mII sample is genetically very similar to the other mII samples, it is clearly isolated geographically and genetically from these, and as a consequence was kept separate.

5.3.10 Phylogeny.

There was insufficient genetic variation among the samples for an exhaustive cladistic analysis to be carried out. However given that

the tII karyotype is clearly ancestral to the fusion races, it is reasonable to assume, on the basis of the distribution of the AAT2 A allele and the aldolase B allele mentioned above and the absence of the aldolase C allele from the Tasmanian and Queensland tII samples, that all of the other groups share a more recent common ancestor with each other than with the Queensland and Tasmanian tII populations.

As was discussed earlier, the UPGMA and Distance Wagner methods will only yield an accurate approximation to the true phylogeny if it is assumed that the rate of protein evolution is constant. Furthermore, from the estimates of sampling error derived from the random samples above, branching points separated by 0.005 Nei units or 0.04 Rogers units cannot be taken too seriously even if a constant rate of protein evolution has occurred. While accepting these limitations and recognising that they are major, in the first instance the implications of the trees derived are discussed below as if they are a true reflection of phylogeny.

The Canberra hybrids were not included in these analyses, since they are considered to be artificially derived and their presence may increase the distortion of the trees.

(a) UPGMA Trees.

(a) Using Nei's (1978) distance measure (Figure 5.3). According to this representation, the Tasmanian tII population was genetically isolated from the mainland population before the other races diverged from each other. The Queensland tII population is a sister group to the Victorian tII and fusion races. Assuming no chromosome fissions have occurred, the position of the Victorian tII population would suggest that the process which caused the fusions arose or was triggered independently in at least three separate lineages - one giving rise to the CV and CIX races, another producing the Perth CIII

race and a third for the mII race. Alternatively, the fusion races may all have a common ancestor but the Victorian tII population clusters with the mII and CIII races because of subsequent gene flow between it and the geographically adjacent mII race. Proximal chiasmata in hybrids of these two races would result in the swapping of large amounts of genetic material between the telocentric and metacentric chromosomes, and so genic introgression is possible without an accompanying introgression of the chromosome types.

The position of the CV and CIX races does not support a hybrid origin with the mII and CIII races as parental to either. Hybridisation between similar races which have not been collected or are extinct is still possible, however.

The clustering together of the CV and CIX races can be explained by all of the the origin models discussed in the last chapter. Under the hybridisation model, the two races would be expected to cluster together if they shared a common parent, or if one were parental to the other. Under the SI model, their proximity can be explained by assuming that the CV race represents an intermediate stage in the formation of the CIX race. Finally, if the CPF model is correct, their close similarity could be attributed to a recent but independent origin of the two races from the same telocentric population. Assuming that the two races can hybridise, it is also possible that they have remained similar through gene flow between the two races arising at the zone of contact in mid NSW (Figure 4.78).

(b) Using Rogers' (1972) distance measure (Figure 5.4). It is difficult to compare the goodness of fit of this tree with that derived from the Nei distance measure because, although the cophenetic correlation is lower, so is the percent standard deviation.

The branching pattern of this tree is essentially the same as that of the the tree discussed above, except that the mII and CII races form a clade, independent of the Victorian tII population. Thus the fusion process need only have occurred in two lineages to have resulted in a phylogeny as shown. Again the two mII samples cluster together, as do the CV and CIX races.

(b) Wagner trees.

Two different rooting procedures were used for the derivation of these trees.

(a) All populations, midpoint rooting (Figure 5.5). This tree shows a similar branching pattern to the Rogers' UPGMA except that the Queensland tII sample clusters with the mII and CIII samples. This is unlikely to be a true representation of the phylogeny, since this sample associates more closely with the mainland mII sample than does the Kangaroo Island mII sample. Even if these two mII populations were derived independently, the geographic distributions would suggest a closer relationship between the mII samples than with the Queensland sample. This also contradicts the conclusion drawn from the aldolase data that Queensland and Tasmanian tII populations are sister groups to the other races.

Other than this, at the higher levels the clustering is the same as both of the UPGMA trees, with the Victorian tII,mII and CIII samples forming one cluster and the CV and CIX races another.

(b) Tasmania tII excluded, outgroup rooting to Queensland tII sample (Figure 5.6). In this example, the Tasmanian tII was excluded because it was based on only one individual, and the tree was rooted to the Queensland tII sample. This was done because the tII race carries the ancestral karyotype, and the Queensland sample clustered as a sister group to all of the other groups except Tasmania in the UPGMA trees.

This tree requires that the fusion process has occurred independently at least three times - in the mainland mII population, the Kangaroo Island and CIII cluster, and again in the CV and CIX cluster. Despite their close geographical proximity, the Kangaroo Island sample clusters with the CIII sample rather than the other mII sample. Given the small genetic distance between these three samples (Table 5.7) and the small size and unknown origin of the Kangaroo Island sample, this branching pattern should not be taken too seriously. Furthermore, although the evidence for constant rates of protein evolution is scant, this configuration implies that protein evolution of the Kangaroo Island population has been extremely retarded in comparison with all of the other populations. This tree also differs from the previous three trees in that the Victorian tII sample clusters with the CV and CIX rather than the CIII and mII groups.

In all of the trees derived in this study, the CV and CIX race which, due to their large sample sizes are assumed to give the most reliable estimates of genetic distance, always appear as sister groups. Another cluster that is stable, except in the final tree, consists of the Victorian tII, CIII and mII samples. The Tasmanian tII individual is always grouped separately from all of the others, and while this is based on only one individual, the comparative magnitude of its genetic distance from all of the other groups (Table 5.7) suggests that this is probably a realistic representation of the genetic relationships. Since this individual also carries the ancestral karyotype morphology, this is also considered to be a realistic representation of its phylogenetic affinities.

5.4 GENERIC RELATIONSHIPS.

Sufficient resources were available to carry out a limited analysis of the genetic relationships between the sparassid genera. Specimens of *Selenops australiensis* were used as an outgroup.

5.4.1 Samples.

Table 5.9 shows the number of species collected and their localities. Preparations from *Pediana* species did not produce adequate results. The following sixteen loci were scored for all of the genera : GP1, GP2, GP3, HK, AK, FUM, LDH, GAPD, GPD, MDH1, MDH2, PGD, Gd, ALD, GPI, PGM, AAT1 and AAT2. The raw data are shown in Appendix 2.

5.4.2 Genetic distances and similarity.

Table 5.10 shows the genetic distance and similarity measures calculated for the genera. Nei's I (similarity) values between the sparassid genera range from 0.498 to 0.664 which is similar to the range reported to occur between closely related genera of other groups (Ayala 1975). The similarity of *S. australiensis* to the other genera is consistently lower than this. The sample sizes are not considered to be large enough to comment on polymorphism and heterozygosity levels.

5.4.3 Phylogeny.

Table 5.11 shows the alleles present at each locus in the genera examined. Given the levels of polymorphism apparent, a cladistic analysis of this data would be misleading since the absence of an allele in any of the genera may well be due to the small sample sizes. However, UPGMA and Wagner trees were generated (Figures 5.7 to 5.9). For the Wagner tree, *Selenops australiensis* was specified as the

outgroup. All of the trees showed identical branching patterns, with *Heteropoda* as a sister group to the other genera and *Isopoda* and *Olios* clustering together. the Wagner tree, which has a higher cophenetic correlation and a lower % S.D. than the two UPGMA trees differs in that the *Heteropoda* branch is closer to the sparassid/*Selenops* node. Given the small distance between the sparassid node and the *Selenops* arm of the tree, this should be viewed as an unresolved trichotomy. That the branching patterns of these trees are a true representation of the phylogeny of this group is supported by morphological and geographic data. Neither *Selenops* nor *Heteropoda* are specific to or most speciose in the Australian region, and both possess a simple palpal morphology in the male. In contrast, the other genera are all endemic or have distributions that centre on Australia, and all possess the complex, spiral-type palpal morphology mentioned in Chapter 1, which is characteristic of many Australian species (Hogg 1902). Thus it is probable that *Selenops* and *Heteropoda* arose elsewhere, while the genera *Delena, Olios* and *Isopoda* represent a more recent, Australian radiation. This study is inconclusive because sample sizes are small and many other sparassid genera exist. However if future work supports the relationships shown in Figure 5.9, family status for *Heteropoda* may be warranted.

5.5 DISCUSSION

5.5.1 Colony structure.

The data on colony structure are surprising because they demonstrate that colonies of *D. cancerides* are not family groups. Even if the size groups examined had representatives from more than one egg sac, the size similarity implies that the egg sacs must have been laid

over a short space of time. Thus, two possibilities exist: either the juveniles migrate between colonies, or gravid females do. The latter is considered more likely, since small solitary juveniles are rarely found whereas solitary adult females are relatively common. Furthermore, in other social spider species such as *Anelosimus eximius, Acharanea wau* and *Diaea socialis* it is the females that migrate to form new colonies, and the males either die before migration occurs or remain with the original colony (Lubin and Crozier 1985, Smith 1986, Main 1987). In these species, however, inbreeding is the rule, and females do not mate with unrelated males. If there is selection for outbreeding in *D. cancerides*, it is logical for females to be the mobile gene vectors, since they are larger than both males and juveniles and so may well have a reduced risk of predation. Three major advantages can be gained from this behaviour:

(i) the female is not "putting all her eggs in one basket". Colonies are prone to sudden and total extermination from agents such as large predators, infectious disease, parasitism and strong winds. By laying eggs in a number of different colonies, the female increases the chance that at least some of her offspring will survive to adulthood.

(ii) the offspring will be able to find a mate easily. Since colonies consist of many individuals of similar size, on maturing, the juveniles will be surrounded by many individuals at a similar stage of maturity. Furthermore, many of these will be unrelated and so levels of inbreeding will be relatively lower.

(iii) predators large enough to prey on small juveniles may be deterred by the larger juveniles.

(iv) the female is assured of a mate. Adult males are often found in colonies, and mating can take place immediately after laying.

It was reported in section 3.2.1 that colony members are often intolerant of conspecifics from other colonies, which suggests that kin-selection is a factor in colony formation and maintenance. Thus the fact that colonies do not appear to be kin groups presents an interesting paradox for which there is no obvious explanation. The aggressive behaviour of juveniles is unlikely to be a barrier to female migration however, because there is no concerted colony defence and older adult females are much larger than juveniles.

Outbreeding in *D. cancerides* may explain the fact that the polymorphism levels observed in this study are much higher than those of *Anelosimus eximius* and *Acharanea wau* (see Table 1.4), which consistently inbreed and disperse regularly.

5.5.2 The aldolase locus : sex-linkage, null alleles and pattern variation.

The aldolase locus is of particular interest since it shows all three of the characteristics listed above, and none of these was observed in any of the other systems.

The pattern variation is clearly influenced by some external factor which causes differential activity in the multimers, but until this factor can be identified, any suggestion of an adaptive significance would be purely speculative. The test for a temperature effect was not conclusive, because it was carried out at only two temperatures and further tests need to be devised to verify this negative result. Differential temperature-related changes in isoenzyme activity have been observed in trout (Baldwin and Hochachka 1970, Moon and Hochachka 1971), but in this case more than one locus is involved and these are turned on and off by ambient temperature levels.

While the pattern of variation observed in the aldolase heterozygotes of *D. cancerides* may be related to some selective advantage, it could not have been responsible for the evolution of the complex sex-linked fusion heterozygosity since this locus is not sex-linked in the CV and CIII populations. It is also unlikely that selection would favour the fixation of sex-linkage of nine chromosomes for the sake of one locus, or even for the sex-linkage of all of the genetic material on the single pair of arms which do not recombine in the chain-of-nine. If the sex-linkage of aldolase does carry a selective advantage, it is more likely that this was a fortuitous event, or that selection has taken advantage of the presence of complex sex-linked fusion heterozygosity to effect sex-linkage.

The sex-linkage of aldolase in the CIX race and the presence of both of the alleles in the CV race is consistent with the hybridisation model. Assuming no gene flow has occurred between the chain carrying races and the tII race, the fact that both the B and C alleles are present in the tII populations (B in Queensland and Tasmania and C in Victoria) which exhibit the ancestral karyotype morphology suggests that the alleles were present before the evolution of these two chain-carrying races. Thus if hybridisation occurred between fusion races derived from the B-carrying and C-carrying tII populations, both would be present in the resulting chain races, as is the case. Furthermore, if recombination was restricted between the two resulting complexes, sex-linkage as seen in the CIX race would result.

In monomeric systems used in this study it is quite possible that null alleles were present but were not detected. It is of interest however that despite the fact that many of the loci examined were dimeric or tetrameric, aldolase was the only system in which a null allele was evident. The null allele detected was presumably on the Y-complex because it occurred in conjunction with the C allele which is

rare or absent on the Y-complex. If the null allele was also present on the X-complex, it would rarely have been detectable since, from the banding patterns of the C/null heterozygotes, it was clear that the null allele has a similar mobility to the B allele and so B/null heterozygotes would have been scored as BB homozygotes. For theoretical reasons, however, it is most likely that the null allele occurs only or most frequently on the Y-complex. In the absence of recombination between sex-linked chromosomes, degeneration of Y-linked chromosomes is not unexpected, since these only occur in the presence of an X-chromosome (Bull 1983). Thus, provided an organism is capable of dosage compensation (if necessary), parts of the Y-linked chromosomes which do not recombine can degenerate totally with no adverse effect. If the null allele observed in the CIX race does represent the commencement of a complete breakdown of the non-recombining Y-linked material, it is still at a very early stage, because the null allele is rare and it does produce a protein product as indicated by the multiple bands of the BC heterozygotes.

5.5.3 Electrophoretic evidence for the origin models.

The three models discussed in Chapter 4 predict different electrophoretic patterns in the chain carrying races.

Under the CPF model, the fusion races arose from a small number of surviving individuals after a catastrophic fusion event that eliminated the bulk of the population. Such a bottleneck may result in the loss of a large number of alleles and a marked change in the frequency of the remaining alleles in the fusion races. Thus initially the fusion races may all have a lower number of alleles than the telocentric races.

The SI model would predict an initial reduction in allele number in the chain carrying races since they arose by the successive

fixation of single fusions, and so each intermediate stage must have arisen from a single individual. Fusions adding to the Y-complex would not require as severe a bottleneck as fusions that added to the X-complex because males with a *de novo* addition to the Y-complex would still breed true with the females, which do not possess a Y-complex. Thus the reduction in allele number may not be as drastic as that expected from the CPF model.

In contrast, if the chain races were produced by hybridisation, they would possess alleles from both parents, and so a larger number of alleles would be expected in the chain carrying races.

While all three models predict a change in the levels of polymorphism, stochastic processes and the generation of novel alleles would result in a return of allele number to levels dictated by population size, breeding systems and other biological parameters.

The fact that the chain races show raised polymorphism levels supports the hybridisation model. The difference in polymorphism levels between the two chain races can be attributed to three possible factors:

(i) the parent races of the CIX race may have held fewer alleles in common than the parents of the CV race which would result in a more polymorphic hybrid gene pool in the CIX race.

(ii) the CV race may itself be parental to the CIX race, so the CIX race is actually made up of a combination of three gene pools.

(iii) the CIX race may be of more recent origin than the CV race and so fewer alleles have been lost by chance.

Other factors may also have been responsible for this observed difference, such as the polymorphism levels in the gene pools of the parent races and the number of individual parents from which each race arose.

As has already been discussed, the branching patterns of the derived trees can be accounted for by all three of the origin models. The fact that the trees suggest that the fusion process has been initiated at least twice and perhaps three times or more is particularly supportive of the CPF model however. If only one initiation of the fusion process had occurred, it could be argued that the fusions arose and were fixed individually over a long period and that all of the fusion races extant today are end points of this single occurrence. That it has occurred on more than one occasion weakens this argument, since it must be argued that in *every case* there has been sufficient time for the process to run to completion and no intermediate phenotypes remain. When this is considered in conjunction with the other cases of wholesale centric fusion in spiders discussed in section 1.4.1, it is more parsimonious to conclude that the accumulation of centric fusions in the spiders has been rapid. The most important question remaining is "*how* rapid?"

5.6 SUMMARY

Using electrophoretic techniques on juveniles and adults collected from the same colony it was demonstrated that colonies of *D. cancerides* are not close family groups; offspring within presumed cohorts were not full sibs and adults from the same colony could not be parental to the juveniles, even if multiple matings have occurred. One explanation for this is that adult females migrate between colonies. Female migration has been observed in other social spiders. Consequently, the reason for intercolony aggression is obscure.

Analysis of twenty loci in samples from the five chromosomal races showed low genetic divergence between all of the groups except

for one individual from Tasmania. Higher polymorphism levels than in other social spider species were attributed to higher levels of outbreeding, based on the cohort data. Only one fixed difference, for the enzyme aldolase, was observed between any of the samples. Aldolase also showed sex-linkage in the CIX race, variable staining patterns in heterozygotes, and a comparatively high frequency of null alleles apparently confined to the Y-complex in the CIX race. The null alleles may represent a more general breakdown of the Y-linked genetic material. The CIX sample was more polymorphic (and consequently more heterozygous) than any of the other races, and polymorphism was also high in the CV race. This is considered to constitute evidence for the hybridisation model.

Despite the lack of sufficient variation for cladistic analysis, some conclusions regarding phylogeny were drawn from phenetic analyses. Although different algorithms were used, there was general agreement between the trees on the constitution of the major groupings. The tII populations from Tasmania appear to be sister groups of the Victorian tII and fusion races and the CV and CIX samples consistently clustered together to the exclusion of the other groups. The placement of the Victorian tII sample implies that the fusion process has arisen on at least two occasions and possibly three or more. The fact that karyotypic fusion has run to completion in all lineages suggests that the fusion process in *D. cancerides* was rapid.

A phenetic analysis of four sparassid genera and the outgroup *Selenops australiensis* placed *Isopoda, Olios* and *Delena* together as a sister group to *Heteropoda*. Considered in conjunction with gross palpal morphology and distributional data, it was suggested that *Isopoda, Olios* and *Delena* represent part of an endemic radiation centred on Australia. The possibility that *Heteropoda* may deserve family status should be considered in future studies on this group.

COLONY 1. (Toukley NSW) Single cohort, carapace width: 2.0 - 2.5mm

	ALD	GPI	PGM	MPI	GOT-1	GOT-2
MOTHER	BB	AA	BB	CC	CC	BB
JUV 1.	AB	AA	BB	CC	CC	BB
JUV 2.	AB	AA	BB	CC	CC	BB
JUV 3.	AB	AA	BB	CC	CC	AB
JUV 4.	AB	AA	BB	BC	CC	AB
JUV 5.	AB	AB	BB	BC	CC	BB
JUV 6.	AA	AA	BB	CC	CC	BB
JUV 7.	AB	AA	BB	AB	CC	AB
JUV 8.	AB	AA	BB	BC	CC	AB
JUV 9.	AB	AB	BB	BC	AC	AB
JUV 10.	AB	AA	AB	BC	CC	AB
father	(*)	(AB)	(AB)	(*)	(AC)	(AB)

COLONY 2. (Toukley NSW) Single cohort.

	ALD	IDH-1	GOT-1	MPI
MOTHER	AA	AA	AB	BB
JUV 1.	AA	AA	AB	BB
JUV 2.	AB	AB	AB	AA
JUV 3.	AA	AB	AA	AB
JUV 4.	AB	AA	AB	BB
JUV 5.	AB	AA	AB	BB
JUV 6.	AA	AA	AB	BB
JUV 7.	AB	AB	AA	AA
JUV 8.	AA	AB	AA	AA
JUV 9.	AA	AA	AA	AA
father	(AB)	(AB)	(AA)	(*)

COLONY 3. (Tamworth NSW) Single cohort.

	PGD	ALD	PGI	PGM	IDH-1	GOT-1
MOTHER	CC	BC	FF	EE	BB	EE
AD. MALE	CC	BB	FF	EE	CC	EE
JUV 1.	CC	BC	FF	EE	BC	DE
JUV 2.	CC	BB	FH	DE	BC	DE
JUV 3.	CC	BC	FF	DD	BB	EE
JUV 4.	CC	BB	FF	EE	BC	EE
JUV 5.	BC	BC	FH	DE	BB	EE
JUV 6.	CC	BC	FF	EE	CC	EE
JUV 7.	CC	BC	FI	EE	BB	EE
JUV 8.	CC	CC	CF	EE	BB	EE
father	(BC)	(BC)	(*)	(*)	(*)	(DE)

Table 5.1 Genotypes of juveniles from within a single cohort, and adult females from three colonies. Possible genotypes of the male parent have been assigned on the assumption that the adult female is the sole female parent. It is clear that this assumption is not borne out in any of the colonies, however. The symbol "*" indicates cases where no possible male genotype can be assigned. Cohort membership was estimated by similarity in size and obvious discontinuity from the next highest and next lowest size group. Only variable loci are shown.

Table 5.2 Specimens of *D. cancerides* studied electrophoretically, separated by sex and race. Locality numbers refer to those given in Figure 4.78. juv = juvenile (and hence sex could not be determined).

RACE	NO. OF INDIVIDUALS (male)	(female)	(juv)	(total)	LOCALITY
tII (northern)	4	3	-	7	1
	2	5	3	10	2
	-	3	-	3	3
TOTAL	**6**	**11**	**3**	**20**	
tII (southern)	1	1	-	2	19
	3	1	-	4	20
	2	2	-	4	21
	2	-	1	3	22
	1	-	-	1	17
TOTAL	**9**	**4**	**1**	**14**	
tII (Tas.)	**1**	**0**	**0**	**1**	36
mII (SA)	1	2	-	3	30
	3	6	-	9	31
	2	-	-	2	35
TOTAL	**6**	**8**	**0**	**14**	
mII (Vic)	4	1	-	5	23
	1	5	-	6	24
	-	1	-	1	25
	2	2	-	4	26
	2	2	-	4	27
	2	2	-	4	28
	1	-	-	1	29
TOTAL	**12**	**13**	**0**	**25**	
mII (K.I.)	**1**	**4**	**-**	**5**	34
CIII	**-**	**5**	**3**	**8**	Perth WA
CV	4	3	-	7	5
	1	1	-	2	6
	1	5	-	6	7
	11	14	-	25	8
	4	1	-	5	9
	1	1	-	2	10
	1	-	-	1	11
	-	2	-	2	Cudal NSW
TOTAL	**23**	**26**	**1**	**50**	
CIX	5	2	-	7	12
	2	-	-	2	13
	-	2	-	2	14
	1	-	-	1	15
	6	2	-	8	16
	36	26	-	62	17
	2	-	-	2	18
	4	-	-	4	32
	1	-	-	1	Roy. Nat. Pk. NSW
	1	-	-	1	Jervis Bay. ACT
	-	1	-	1	Wagga Wagga. NSW
	-	1	-	1	Brindabella Rge. ACT
TOTAL	**58**	**34**	**0**	**92**	
HYBRID POP.	**7**	**9**	**0**	**16**	Canberra ACT
GRAND TOTAL				**244**	

Table 5.3 Allelic frequencies in samples from chromosome races of *D. cancerides*. Sexes separate and pooled for CV and CIX races, and for Canberra hybrid sample.

	POPULATION															
LOCUS	tII (Qld)	tII (Vic)	mII (SA)	mII (Vic)	mII (KI)	CIII	CV (tot)	CV (male)	CV (female)	HYB (tot)	HYB (male)	HYB (female)	CIX (tot)	CIX (male)	CIX (female)	tII (Tas)
GP1																
(N)	20	14	14	25	5	8	50	23	27	16	7	9	92	58	34	1
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GP2																
(N)	20	14	14	25	5	8	50	23	27	16	7	9	92	58	34	1
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GP3																
(N)	20	14	14	25	5	8	50	23	27	16	7	9	92	58	34	1
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
HK																
(N)	20	14	14	25	5	8	50	23	27	16	7	9	92	58	34	1
A	0.000	0.000	0.000	0.020	0.000	0.000	0.010	0.000	0.019	0.000	0.000	0.000	0.005	0.009	0.000	0.000
B	1.000	1.000	1.000	0.980	1.000	1.000	0.960	0.978	0.944	1.000	1.000	1.000	0.973	0.966	0.985	1.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.022	0.037	0.000	0.000	0.000	0.011	0.009	0.015	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.017	0.000	0.000
AK																
(N)	20	14	14	25	5	8	50	23	27	16	7	9	91	57	34	1
A	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.022	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	1.000	1.000	0.980	0.978	0.981	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FUM																
(N)	20	14	14	25	5	8	40	19	21	16	7	9	92	58	34	1
A	0.025	0.000	0.000	0.000	0.000	0.000	0.025	0.026	0.024	0.000	0.000	0.000	0.022	0.009	0.044	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.111	0.016	0.026	0.000	0.000
C	0.825	1.000	1.000	0.980	1.000	1.000	0.975	0.974	0.976	0.906	0.929	0.889	0.962	0.966	0.956	1.000
D	0.150	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.031	0.071	0.000	0.000	0.000	0.000	0.000
LDH																
(N)	20	14	14	25	5	8	48	23	25	16	7	9	92	58	34	1
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.969	1.000	0.944	0.935	0.931	0.941	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.056	0.065	0.069	0.059	0.000
GAPDH																
(N)	20	14	14	25	5	8	50	23	27	16	7	9	92	58	34	1
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPD																
(N)	20	14	14	25	5	8	50	23	27	16	7	9	92	58	34	1
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.015	0.000
B	0.000	0.036	0.071	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.009	0.044	0.000
C	1.000	0.964	0.929	1.000	1.000	0.750	0.990	1.000	0.981	0.969	1.000	0.944	0.951	0.974	0.912	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.019	0.031	0.000	0.056	0.022	0.017	0.029	1.000
MDH1																
(N)	20	14	14	25	5	8	20	10	10	16	7	9	91	58	33	1
A	0.050	0.000	0.143	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.027	0.026	0.030	0.000
B	0.950	1.000	0.857	0.980	1.000	1.000	0.975	0.950	1.000	0.938	0.857	1.000	0.934	0.948	0.909	1.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.030	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.050	0.000	0.063	0.143	0.000	0.027	0.026	0.030	0.000

MDH2

(N)	20	14	14	25	5	8	20	10	10	16	7	9	92	58	34	1
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.015	0.000
B	1.000	1.000	1.000	1.000	1.000	0.938	1.000	1.000	1.000	0.969	0.929	1.000	0.995	1.000	0.985	1.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.071	0.000	0.000	0.000	0.000	0.000

PGD

(N)	8	6	14	25	5	8	47	23	24	16	7	9	92	58	34	1
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.036	0.000	0.000	0.000	0.011	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	1.000	1.000	0.964	1.000	1.000	1.000	0.968	0.957	0.979	0.906	0.857	0.944	0.929	0.948	0.897	0.500
D	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.043	0.000	0.000	0.000	0.000	0.005	0.009	0.000	0.500
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.094	0.143	0.056	0.065	0.043	0.103	0.000

Gd

(N)	8	6	14	25	5	8	47	23	24	16	7	9	92	58	34	1
A	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.813	0.833	1.000	1.000	1.000	1.000	0.989	0.978	1.000	1.000	1.000	1.000	0.967	0.983	0.941	0.500
C	0.125	0.000	0.000	0.000	0.000	0.000	0.011	0.022	0.000	0.000	0.000	0.000	0.033	0.017	0.059	0.000
D	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500

GPI

(N)	20	14	14	25	5	8	50	23	27	16	7	9	92	58	34	1
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.017	0.029	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
C	0.025	0.036	0.071	0.020	0.000	0.000	0.010	0.000	0.019	0.156	0.071	0.222	0.038	0.043	0.029	0.000
D	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.071	0.000	0.000	0.000	0.000	0.000
E	0.000	0.036	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.031	0.071	0.000	0.005	0.000	0.015	0.000
F	0.900	0.893	0.929	0.840	1.000	1.000	0.930	0.957	0.907	0.750	0.786	0.722	0.848	0.871	0.809	0.000
G	0.075	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.009	0.015	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.022	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.080	0.000	0.000	0.030	0.022	0.037	0.031	0.000	0.056	0.038	0.034	0.044	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.019	0.000	0.000	0.000	0.038	0.026	0.059	0.000

PGM

(N)	20	14	14	25	5	8	50	23	27	16	7	9	92	58	34	1
A	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.009	0.029	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.027	0.026	0.029	0.000
C	0.000	0.107	0.036	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.141	0.155	0.118	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.130	0.037	0.031	0.071	0.000	0.005	0.009	0.000	0.000
E	0.975	0.750	0.821	0.920	1.000	0.688	0.910	0.870	0.944	0.875	0.929	0.833	0.788	0.767	0.824	1.000
F	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.025	0.071	0.107	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.036	0.000	0.000	0.000	0.000	0.010	0.000	0.019	0.094	0.000	0.167	0.022	0.034	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.313	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

IDH1

(N)	20	13	14	25	5	8	48	23	25	16	7	9	92	58	34	1
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.027	0.026	0.029	0.000
B	0.000	0.308	0.036	0.040	0.000	0.000	0.625	0.652	0.600	0.250	0.286	0.222	0.533	0.560	0.485	0.500
C	0.900	0.692	0.893	0.960	1.000	1.000	0.375	0.348	0.400	0.750	0.714	0.778	0.435	0.414	0.471	0.500
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.015	0.000
E	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

IDH2

(N)	20	13	14	25	5	8	48	23	25	8	1	7	61	31	30	1
A	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	1.000	1.000	0.990	0.978	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

AAT1

(N)	20	14	12	25	5	8	50	23	27	13	7	6	92	58	34	1
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.017	0.000	0.000
B	0.850	0.036	0.000	0.000	0.000	0.000	0.040	0.000	0.074	0.000	0.000	0.000	0.011	0.009	0.015	0.000
C	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.083	0.080	0.000	0.000	0.110	0.174	0.056	0.038	0.071	0.000	0.054	0.043	0.074	0.000
E	0.100	0.964	0.917	0.820	1.000	1.000	0.820	0.804	0.833	0.962	0.929	1.000	0.908	0.922	0.882	1.000
F	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.022	0.037	0.000	0.000	0.000	0.011	0.000	0.029	0.000
H	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.009	0.000	0.000

AAT2

(N)	11	8	12	25	5	8	50	23	27	16	7	9	91	57	34	1
A	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.009	0.015	1.000
B	0.955	1.000	1.000	1.000	1.000	1.000	0.960	1.000	0.926	1.000	1.000	1.000	0.989	0.991	0.985	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.074	0.000	0.000	0.000	0.000	0.000	0.000	0.000

ALD

(N)	20	13	14	25	4	8	48	23	25	16	7	9	90	56	34	1
A	0.000	0.000	0.000	0.000	0.000	0.188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.975	0.000	0.250	0.120	0.000	0.625	0.563	0.630	0.500	0.188	0.357	0.056	0.422	0.598	0.132	1.000
C	0.000	0.923	0.714	0.860	1.000	0.000	0.438	0.370	0.500	0.813	0.643	0.944	0.561	0.402	0.824	0.000
D	0.025	0.000	0.000	0.020	0.000	0.188	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.015	0.000
E	0.000	0.077	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.029	0.000

Table 5.4 **(I)** *G*-test comparisons of distributions of absolute frequencies of B and C alleles in males and females in the CIX and CV races of *D. cancerides* for aldolase. (II) Frequencies of alleles B and C on the X- and Y-complexes and *G*-test of the absolute frequencies of the alleles on the X-complex between males and females of the CIX race.

(I)

CIX RACE	ALLELIC FREQUENCY		
	B	C	
MALE	67	45	$G = 36.1^{***}$
FEMALE	9	53	

CV RACE	ALLELIC FREQUENCY		
	B	C	
MALE	29	17	$G = 1.7$ (ns)
FEMALE	25	25	

$$\chi^2_{1,.05} = 3.84,\ \chi^2_{1,.001} = 10.828$$

(II)

CIX RACE	GENOTYPE FREQUENCY		
	BB	BC	CC
MALE	11	45	0
FEMALE	2	5	24

FREQ. OF ALLELE B ON X-COMPLEX (from females) = 9/62 = 14.5%
FREQ. OF ALLELE C ON X-COMPLEX (from females) = 53/62 = 85.5%
FREQ. OF ALLELE B ON Y-COMPLEX = 100%
FREQ. OF ALLELE C ON Y-COMPLEX = 0%
FREQ. OF ALLELE B ON X-COMPLEX (from males) = 11/56 = 19.6%
FREQ. OF ALLELE C ON X-COMPLEX (from males) = 45/56 = 80.4%

	ABSOLUTE FREQUENCY ON X-COMPLEX		
	B	C	
MALE	11	45	$G = 0.58$ (ns)
FEMALE	9	53	

	CIX	CV (n=50)
CIX (n=92)		** (CIX higher)
CIII (n=8)	***	***
mII (SA) (n=14)	***	ns
tII (Vic) (n=14)	***	ns
HYB (n=16)	***	ns
tII (Qld) (n=20)	***	*
mII (Vic) (n=25)	***	**

Table 5.5 Comparisons of allele number between samples. In the CV vs CIX test, the Wilcoxon ranking test (Walpole 1974) was used. In all of the other comparisons, significances were obtained from binomial distribution tables after reconstructing the sampling distributions of the CV and CIX races. Significantly more alleles were detected in the CIX race than in any of the other samples. The CV sample showed significantly more alleles than the CIII, Queensland tII and Victorian mII samples, but significantly fewer than the CIX race. * = probability (p) ≤ 0.05, ** = $p \leq 0.0107$, *** = $p \leq 0.001$

Table 5.6 Percentage of polymorphic loci, mean number of alleles per locus and mean heterozygosity for the *D. cancerides* samples analysed.

POPULATION	MEAN SAMPLE SIZE PER LOCUS	MEAN NO. OF ALLELES PER LOCUS	PERCENTAGE OF LOCI POLYMORPHIC*	MEAN HETEROZYGOSITY	
				DIRECT COUNT	HDYWBG EXPECTED**
tII (Qld)	18.3 (1.0)	1.6 (0.2)	42.1	0.065 (0.021)	0.080 (0.027)
tII (Vic)	12.7 (0.6)	1.6 (0.3)	31.6	0.054 (0.026)	0.080 (0.034)
mII (SA)	13.8 (0.1)	1.5 (0.2)	36.8	0.065 (0.022)	0.068 (0.024)
mII (Vic)	25.0 (0.0)	1.7 (0.3)	36.8	0.046 (0.020)	0.051 (0.023)
mII (Kangaroo I)	5.0 (0.0)	1.0 (0.0)	0.0	0.000 (0.000)	0.000 (0.000)
CIII	8.0 (0.0)	1.2 (0.1)	15.8	0.066 (0.041)	0.052 (0.031)
CV (male)	21.4 (0.9)	1.7 (0.2)	57.9	0.061 (0.023)	0.080 (0.029)
CV (female)	24.3 (1.2)	1.9 (0.3)	52.6	0.048 (0.015)	0.078 (0.029)
Hybrids	15.4 (0.4)	1.8 (0.2)	52.6	0.083 (0.027)	0.094 (0.030)
CIX (male)	56.5 (1.4)	2.6 (0.4)	63.2	0.088 (0.027)	0.098 (0.033)
CIX (female)	33.7 (0.2)	2.5 (0.4)	68.4	0.104 (0.034)	0.124 (0.034)
tII (Tasmania)	1.0 (0.0)	1.2 (0.1)	15.8	0.158 (0.086)	0.158 (0.086)

* A LOCUS IS CONSIDERED POLYMORPHIC IF MORE THAN ONE ALLELE WAS DETECTED

** UNBIASED ESTIMATE (SEE NEI, 1978)

Table 5.7 Genetic distances and identity between population samples of *D. cancerides*.

BELOW DIAGONAL: ROGERS (1972) GENETIC DISTANCE
ABOVE DIAGONAL: NEI (1978) UNBIASED GENETIC DISTANCE

	POPULATION	1	2	3	4	5	6	7	8	9	10	11	12
1	tII (QLD)	*****	0.048	0.044	0.038	0.045	0.055	0.060	0.053	0.048	0.062	0.055	0.275
2	tII (Vic)	0.095	*****	0.004	0.005	0.007	0.010	0.009	0.007	0.002	0.004	0.003	0.213
3	mII (SA)	0.089	0.044	*****	0.001	0.002	0.005	0.020	0.017	0.003	0.015	0.011	0.226
4	mII (Vic)	0.075	0.046	0.032	*****	0.002	0.008	0.022	0.018	0.003	0.017	0.013	0.225
5	mII (KANGAROO I)	0.080	0.044	0.034	0.024	*****	0.007	0.025	0.020	0.005	0.020	0.016	0.229
6	CIII	0.111	0.059	0.049	0.051	0.033	*****	0.032	0.028	0.011	0.025	0.021	0.229
7	CV (male)	0.110	0.059	0.064	0.058	0.064	0.088	*****	0.000	0.011	0.002	0.003	0.221
8	CV (female)	0.103	0.053	0.062	0.051	0.056	0.084	0.028	*****	0.008	0.002	0.002	0.210
9	Hybrids (Canberra)	0.100	0.046	0.048	0.046	0.050	0.070	0.062	0.059	*****	0.007	0.004	0.208
10	CIX (males)	0.113	0.045	0.057	0.056	0.064	0.081	0.037	0.034	0.047	*****	0.000	0.214
11	CIX (female)	0.111	0.051	0.058	0.060	0.072	0.086	0.047	0.045	0.046	0.026	*****	0.204
12	tII (Tasmania)	0.283	0.226	0.249	0.245	0.237	0.251	0.239	0.225	0.233	0.231	0.224	*****

BELOW DIAGONAL: NEI (1978) UNBIASED GENETIC IDENTITY

	POPULATION	1	2	3	4	5	6	7	8	9	10	11	12
1	tII (Qld)	*****											
2	tII (Vic)	0.953	*****										
3	mII (SA)	0.957	0.996	*****									
4	mII (Vic)	0.963	0.995	0.999	*****								
5	tII (KANGAROO I)	0.956	0.993	0.998	0.998	*****							
6	CIII	0.947	0.990	0.995	0.992	0.993	*****						
7	CV (male)	0.941	0.991	0.980	0.979	0.975	0.969	*****					
8	CV (female)	0.948	0.993	0.983	0.983	0.980	0.972	1.000	*****				
9	Hybrids (Canberra)	0.953	0.998	0.997	0.997	0.995	0.989	0.989	0.992	*****			
10	CIX (males)	0.940	0.996	0.985	0.983	0.980	0.975	0.998	0.998	0.993	*****		
11	CIX (female)	0.947	0.997	0.989	0.987	0.984	0.979	0.997	0.998	0.996	1.000	*****	
12	tII (Tasmania)	0.760	0.808	0.798	0.798	0.795	0.795	0.802	0.810	0.812	0.807	0.816	*****

Table 5.8 Genetic distance between random samples of 20 individuals from the CIX race. Aldolase not included.

BELOW DIAGONAL: ROGERS (1972) GENETIC DISTANCE

ABOVE DIAGONAL: NEI (1978) UNBIASED GENETIC DISTANCE

SAMPLE NO.	1	2	3	4	5	6	7	8	9	10
1	*****	0.003	0.005	0.001	0.003	0.001	0.000	0.002	0.000	0.005
2	0.042	*****	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000
3	0.040	0.038	*****	0.002	0.000	0.001	0.000	0.000	0.003	0.000
4	0.032	0.032	0.044	*****	0.000	0.000	0.000	0.000	0.001	0.000
5	0.035	0.043	0.039	0.031	*****	0.001	0.000	0.002	0.001	0.000
6	0.032	0.026	0.037	0.027	0.040	*****	0.000	0.000	0.000	0.000
7	0.029	0.027	0.034	0.026	0.040	0.027	*****	0.000	0.000	0.000
8	0.040	0.031	0.026	0.032	0.048	0.032	0.026	*****	0.000	0.000
9	0.030	0.038	0.040	0.034	0.038	0.031	0.035	0.037	*****	0.003
10	0.039	0.029	0.028	0.028	0.036	0.025	0.029	0.033	0.040	*****

Table 5.9 Sex, sample size and collection localities of species analysed for generic comparisons. Locality numbers refer to those given in Figure 4.78.

SPECIES	NO. OF INDIVIDUALS (m)	(f)	(tot)	LOCALITY
I. tepperi	2	2	4	Canberra, ACT
I. vaster	2	2	4	17
O. diana	4	-	4	Canberra ACT, Trangie NSW
H. procera	1	1	2	Sydney NSW, Brisbane Qld
H. sp. 2	-	1	1	Jakiluka Billabong NT
D. cancerides	-	1	1	14
S. australiensis	3	2	5	1,9,10, Grafton NSW, Darwin NT

Table 5.10 Genetic distance and similarity indices for four huntsman genera and the species *Selenops australiensis*.

BELOW DIAGONAL: ROGERS (1972) GENETIC DISTANCE

ABOVE DIAGONAL: NEI (1978) UNBIASED GENETIC DISTANCE

	POPULATION	1	2	3	4	5
1	SELENOPS	*****	0.956	1.192	1.088	0.731
2	DELENA	0.623	*****	0.494	0.612	0.597
3	ISOPODA	0.667	0.412	*****	0.410	0.430
4	OLIOS	0.666	0.465	0.376	*****	0.696
5	HETEROPODA	0.533	0.483	0.395	0.529	*****

BELOW DIAGONAL: NEI (1978) UNBIASED GENETIC IDENTITY

	POPULATION	1	2	3	4	5
1	SELENOPS	*****				
2	DELENA	0.385	*****			
3	ISOPODA	0.304	0.610	*****		
4	OLIOS	0.337	0.542	0.664	*****	
5	HETEROPODA	0.482	0.551	0.651	0.498	*****

Table 5.11 Alleles present in the four huntsman genera and *Selenops australiensis*.

	GP1	GP2	GP3	HK	AK	FUM	LDH	GAPDH	GPD	MDH1	MDH2	PGD	Gd	ALD	GPI	PGM	AAT1	AAT2
Selenops	A	AB	A	DE	A	A	B	A	A	AB	ABC	B	ABCD	CDE	AF	ABE	D	AC
Delena	C	A	A	C	B	B	C	A	A	B	B	A	G	D	D	G	EG	B
Isopoda	B	A	A	C	B	B	C	A	BD	BCDEF	B	CDE	CDF	E	CEG	DEF	BE	B
Olios	CD	A	A	B	B	B	C	A	C	B	B	E	E	E	E	EF	BCD	B
Heteropoda	B	A	A	BC	B	B	A	A	A	CF	B	BCF	BE	ABD	BF	CEF	ABE	B

Figures 5.1 (upper) and **5.2**(lower) Aldolase gels for CIX and CV races of *D. cancerides*. Aldolase is a tetrameric enzyme, and in both cases the five-banded BC heterozygotes are clearly distinguishable from the single-banded CC and BB homozygotes. In the CIX gel (top), all of the CC homozygotes are female, and the BC heterozygotes are female. In the CV gel (bottom), the sexes were loaded in the same order as in the CIX gel but there is no apparent sex-linkage.

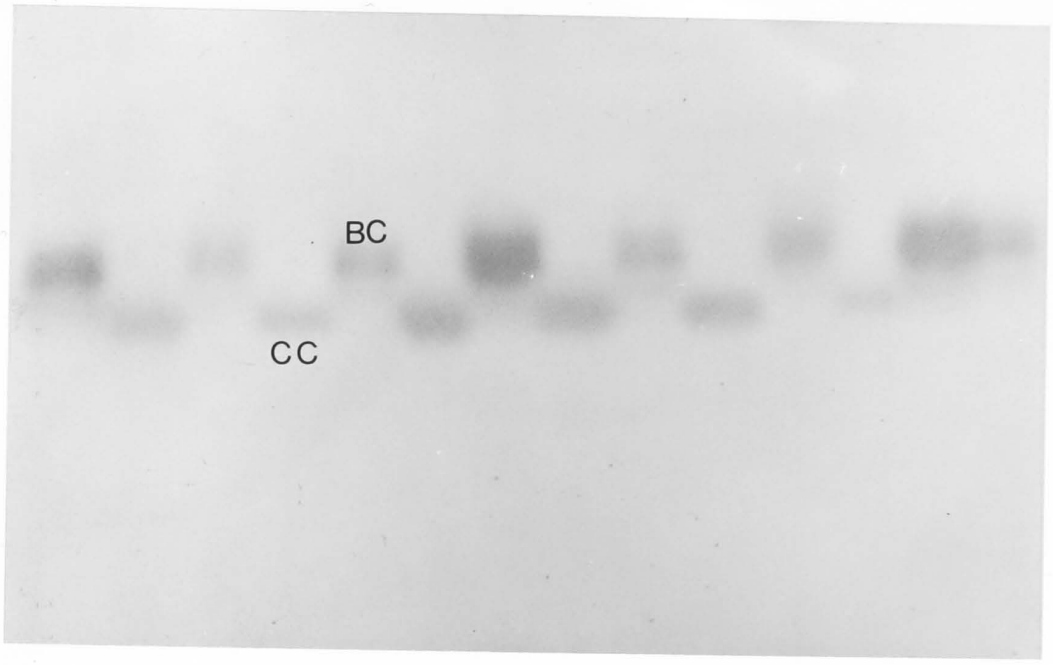
In heterozygotes, if both of the gene products (B and C) were produced in equal quantities and tetramer formation random, the five types of tetrameric molecules formed should be present in the ratio (from top to bottom) of 1 : 4 : 6 : 4 : 1. Thus, assuming equal activity of each tetrameric molecule, staining intensity of these products should follow this pattern, with the middle band the darkest, the top and bottom bands equally pale, and the second and fourth bands intermediate in intensity. However, this was not always the case in BC heterozygotes of *D. cancerides*. In these two gels, both the middle *and* the second top bands stained most intensely. This can be explained in three ways:

(i) the B and C gene products are not produced in equal amounts,

(ii) tetramer formation is not random, or

(iii) the tetrameric molecules do not have equal activity.

Of these, (iii) is considered most probable because this unusual staining pattern was not consistent; when run again, the same samples sometimes gave the more usual pattern, with the middle band the most intense. For this reason it is suggested that the activity of the tetrameric products may be related to environmental factors.


BC
CC

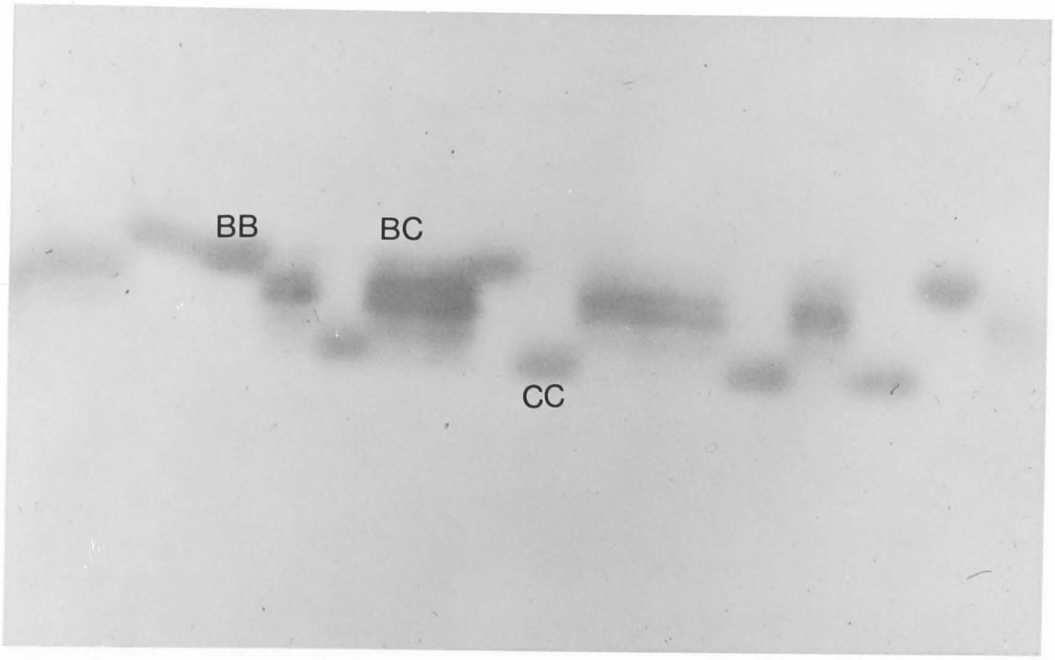

BB
BC
CC

Figure 5.3 UPGMA tree of *D. cancerides* samples, derived using Nei (1978) genetic distance coefficient.

COPHENETIC CORRELATION = 0.947

% S.D. (Fitch and Margoliash 1967) = 74.365

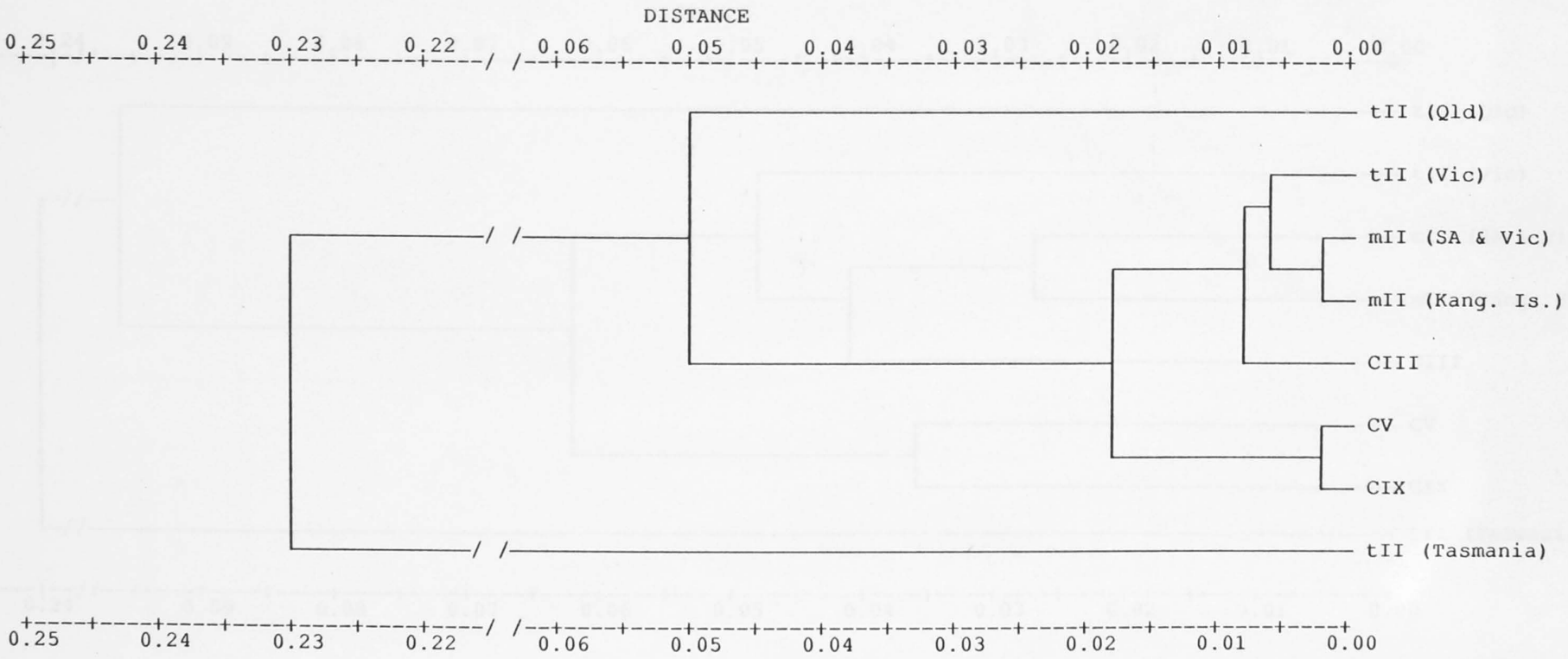


Figure 5.4 UPGMA tree for *D. cancerides* samples, derived using Rogers' (1972) distance coefficient.

COPHENETIC CORRELATION =0.881

% S.D. (Fitch and Margoliash 1967) = 16.109

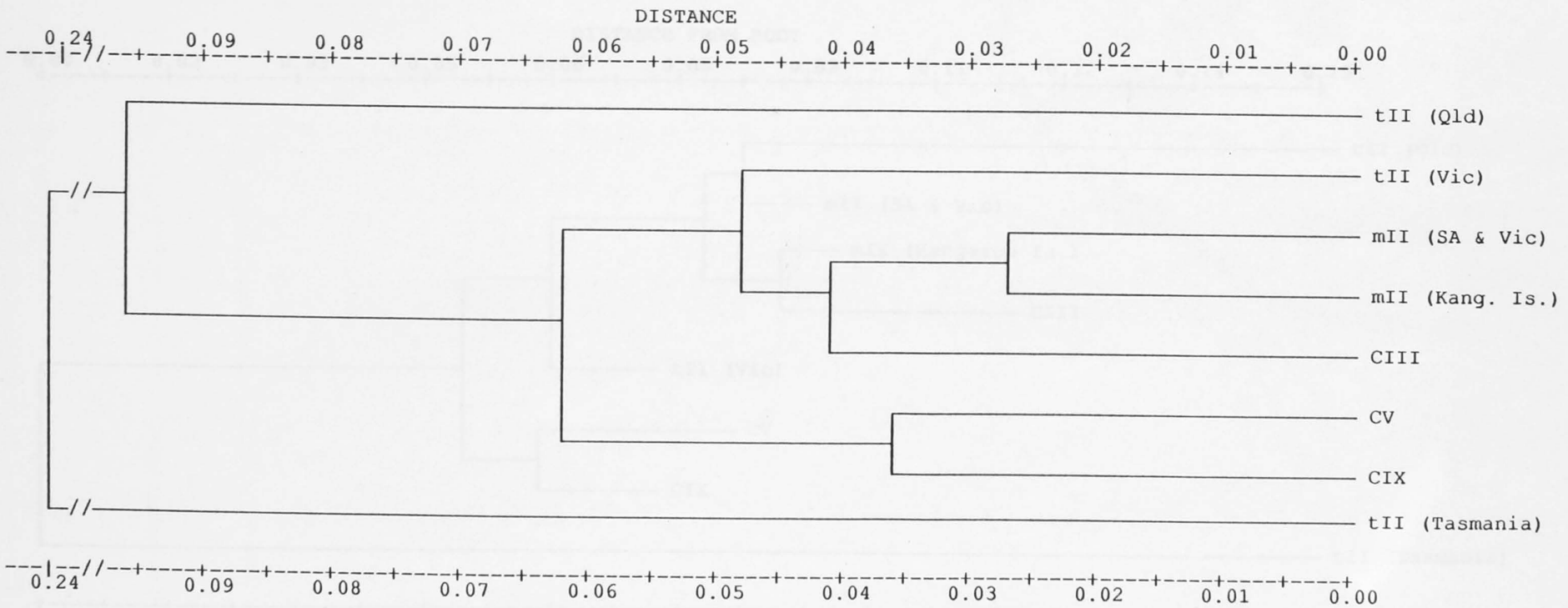


Figure 5.5 Wagner tree for *D. cancerides* samples, derived using Rogers' (1972) distance coefficient and midpoint rooting.
COPHENETIC CORRELATION = 0.995
% S.D. (Fitch and Margoliash 1967) = 13.387

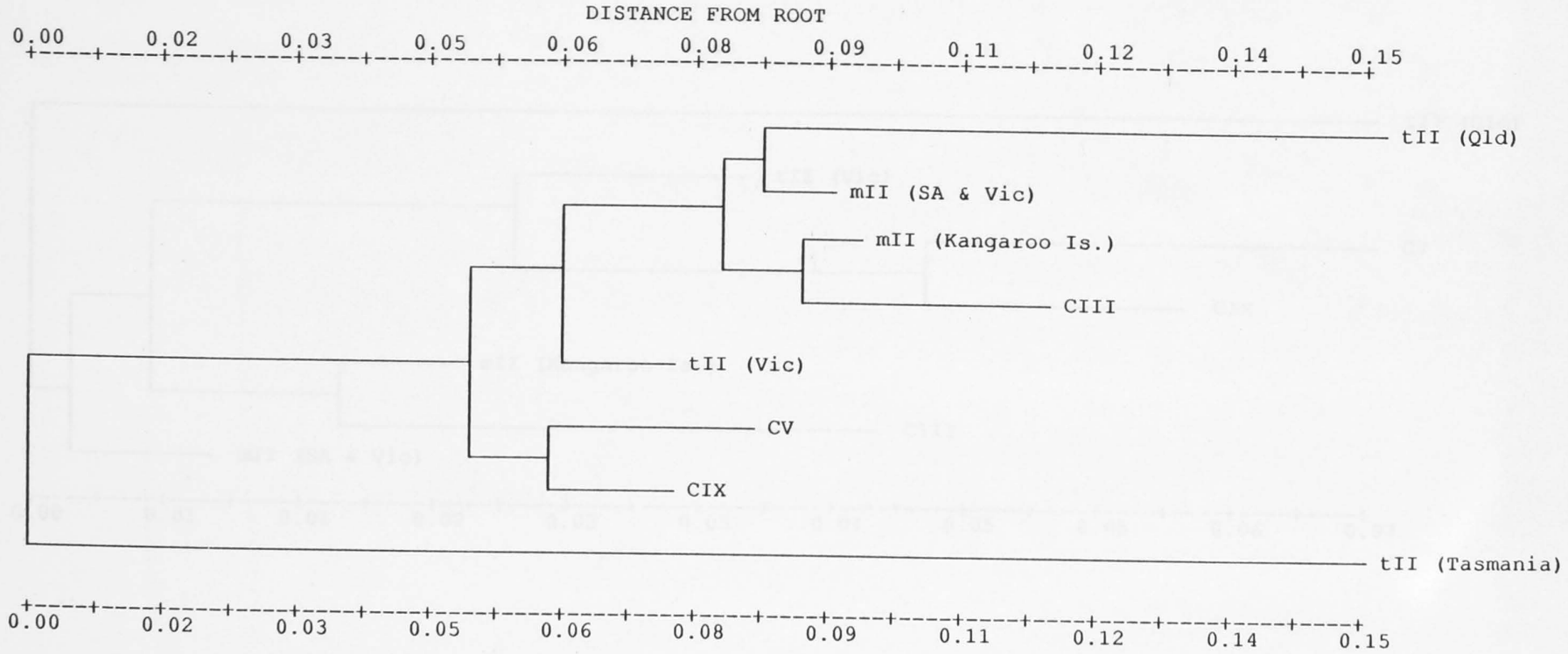


Figure 5.6 Wagner tree for *D. cancerides* samples, derived using Rogers' (1972) distance coefficient and the Queensland tII sample as an outgroup. Tasmanian tII sample not included.

COPHENETIC CORRELATION = 0.968

% S.D. (Fitch and Margioliash 1967) = 15.285

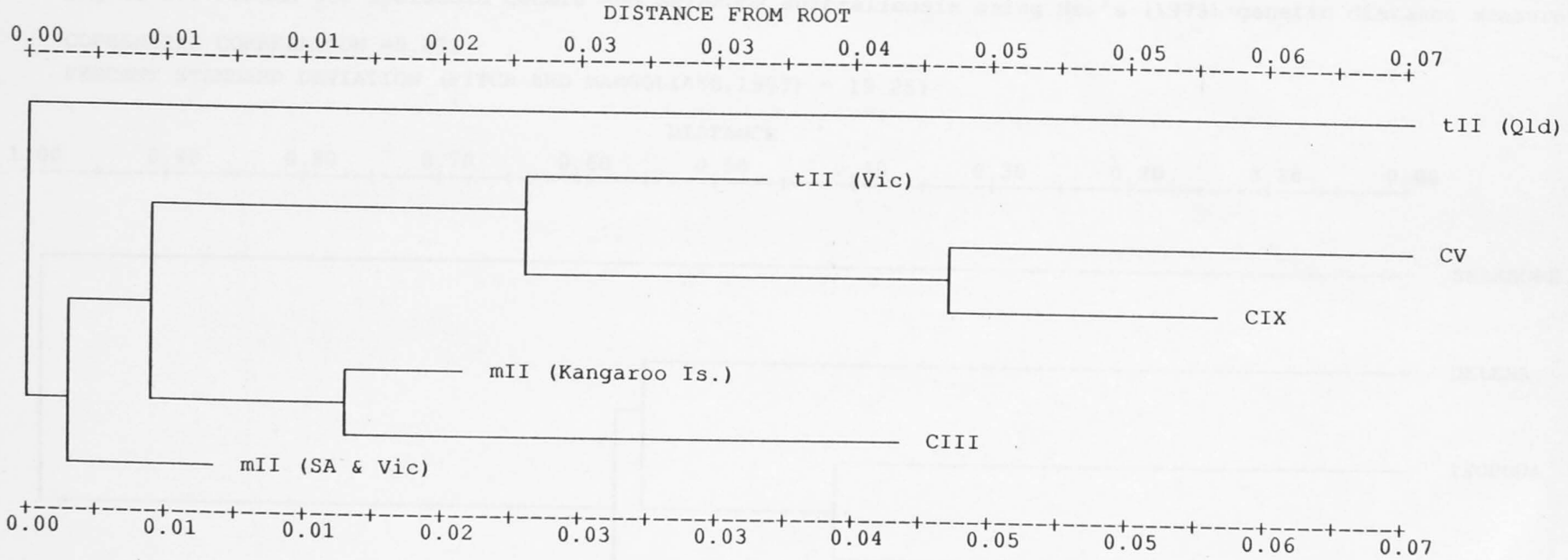


Figure 5.7 UPGMA for sparassid genera and *Selenops australiensis* using Nei's (1978) genetic distance measure.

COPHENETIC CORRELATION =0.871

PERCENT STANDARD DEVIATION (FITCH AND MARGOLIASH,1967) = 19.251

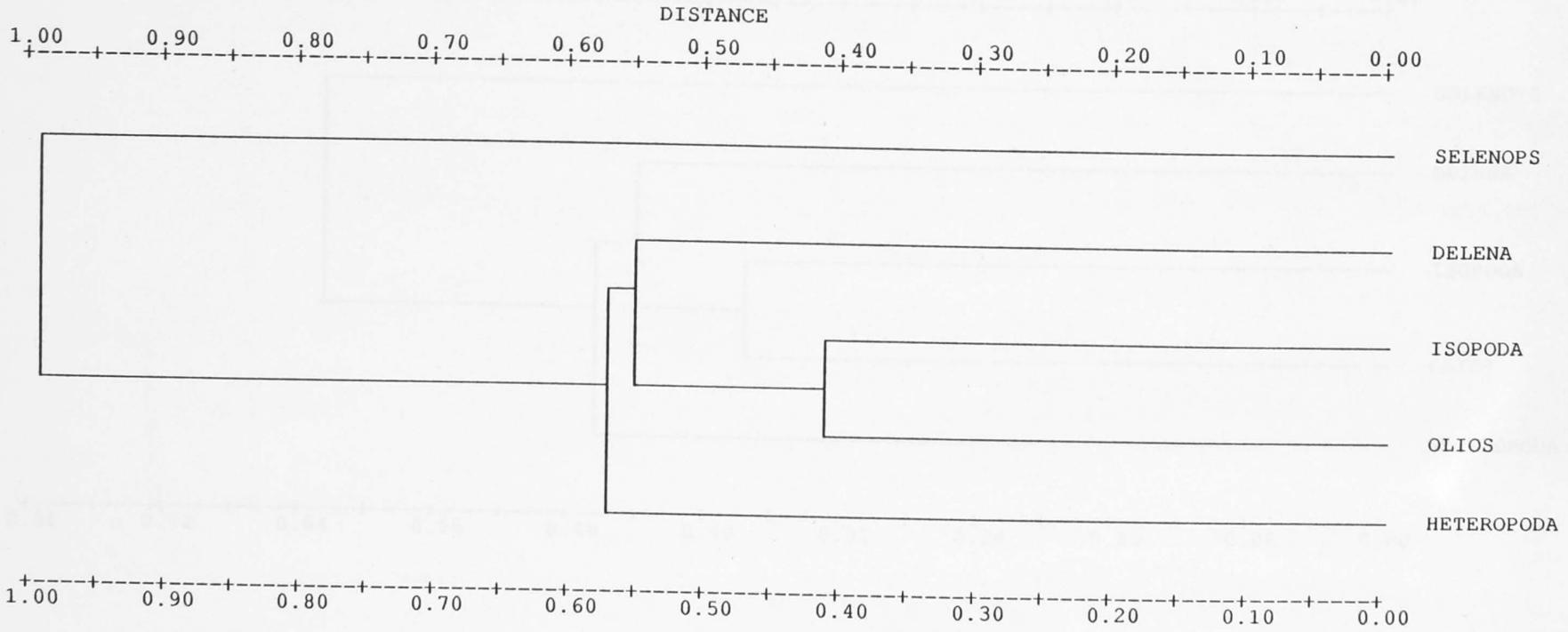


Figure 5.8 UPGMA for sparassid genera and *Selenops australiensis* using Rogers' (1972) genetic distance measure.

COPHENETIC CORRELATION =0.887

PERCENT STANDARD DEVIATION (FITCH AND MARGOLIASH,1967) = 10.170

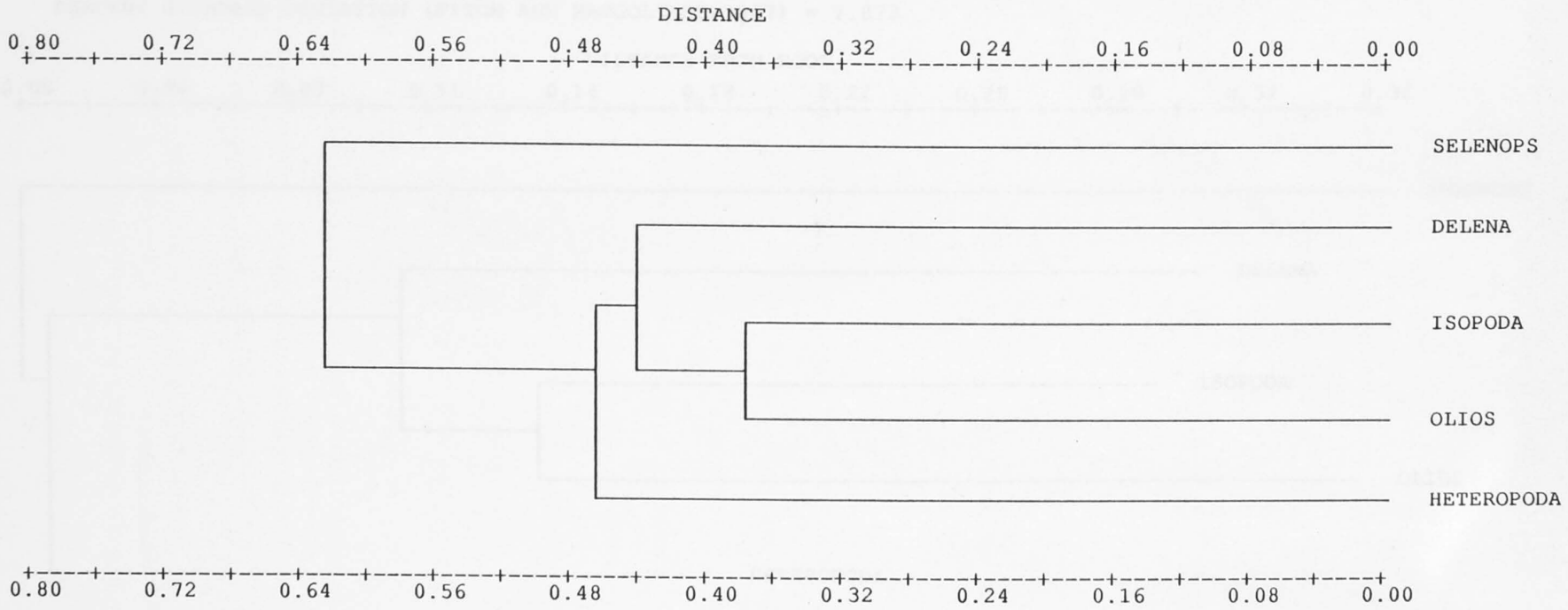
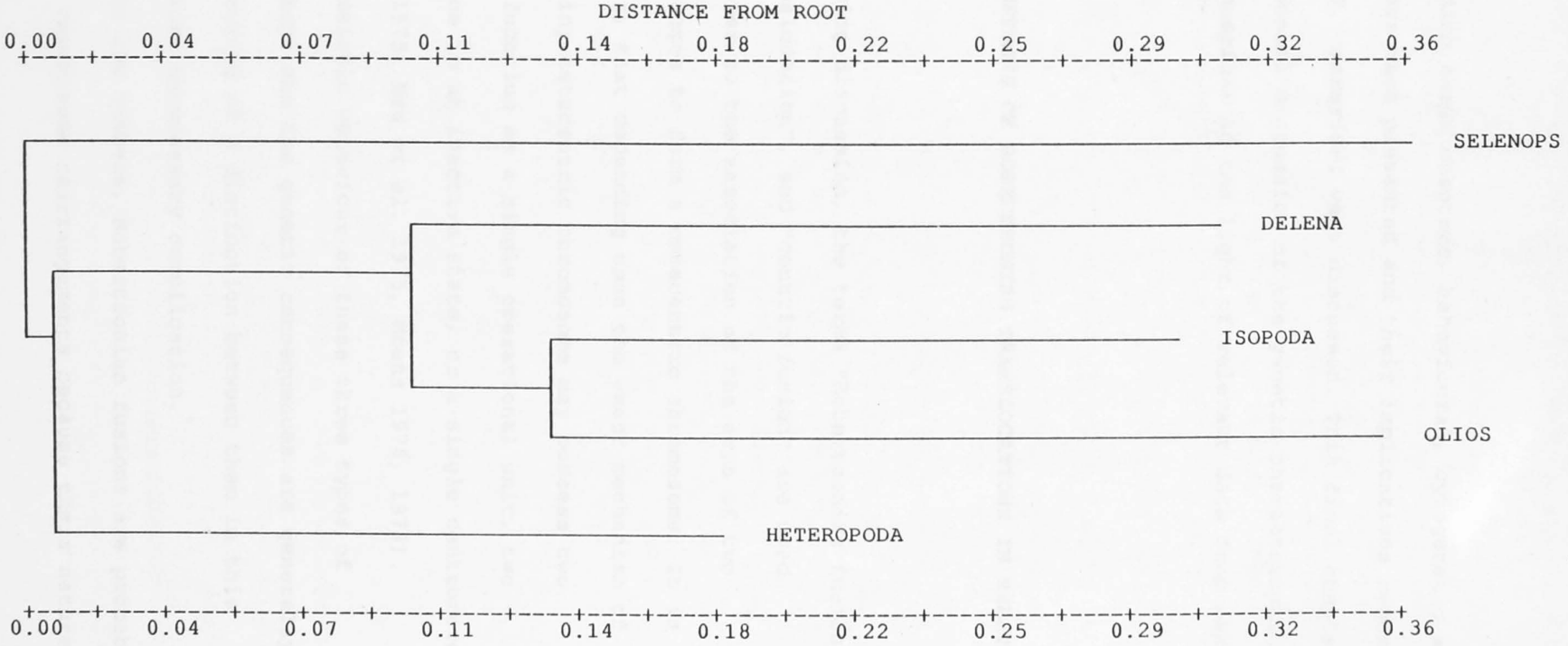


Figure 5.9 Wagner tree for sparassid genera and *Selenops australiensis* using Rogers' (1972) genetic distance measure.

COPHENETIC CORRELATION =0.965

PERCENT STANDARD DEVIATION (FITCH AND MARGOLIASH,1967) = 7.873



CHAPTER 6.

GENERAL DISCUSSION

In the preceding three chapters, behavioural, cytogenetic and electrophoretic data were presented and their implications regarding the evolution of *D. cancerides* were discussed. This final chapter presents a more general discussion of the genetic characteristics observed in *D. cancerides* in the light of relevant data from other organisms.

6.1 THE MEIOTIC EFFECTS OF ROBERTSONIAN TRANSLOCATIONS IN RELATION TO SPECIATION

In the following discussion, the terms "Robertsonian fusion", "Robertsonian translocation", and "centric fusion" are used synonymously to refer to the association of the arms of two telocentric chromosomes to form a metacentric chromosome. It is recognised, however, that depending upon the exact mechanism of fusion, the resulting metacentric chromosome may possess two centromeres which function as a single operational unit, two centromeres with one in an inactive state, or a single centromere (John and Freeman 1975, Hsu *et al.* 1975, Moens 1978, 1979). Nevertheless, the meiotic behaviour of these three types of metacentric chromosome and the genetic consequences are generally the same, and so the drawing of a distinction between them in this discussion would cause unnecessary complication.

When present in low numbers, Robertsonian fusions are probably the most benign of chromosome rearrangements because their nature and

behaviour causes minimal disruption to genetic processes in a cell. For example:

(i) Robertsonian fusion does not disrupt gene sequences or coadapted gene complexes. Other rearrangements such as pericentric and paracentric inversions and translocations of chromosomal segments require chromosomal breakages which may occur within a gene or within a group of genes which have "coadapted" to function as a unit (Dobzhansky 1950). Robertsonian fusions, on the other hand, require breakage only in the centromeric region, either extremely proximal to or within the centromere, or perhaps on the opposite side of the centromere to the chromosome arm (John and Freeman 1975), and there is no evidence that this impairs the function of the centromere in any case. Admittedly, fusions may alter chiasma distributions (White and Chinnick 1957, Hewitt and John 1972, John and Freeman 1975, 1976, Shaw 1981), and altered chiasma positions may well be responsible for hybrid breakdown in the grasshopper *Caledia captiva* (Shaw and Wilkinson 1980, Shaw *et al.* 1982, Shaw and Coates 1983). In general, however, effects of this kind must surely be less disruptive than the production of duplication/deficiency products which result from crossovers within inverted regions or within differential segments of translocation products.

(ii) Fusions are usually meiotically balanced and stable. In the heterozygous state, centric fusions do not often lead to high levels of infertility through incorrect segregation of the resultant trivalent (Capanna *et al.* 1976, Searle 1984, Baker and Bickham 1986). Indeed, even when three trivalents are present in sheep, fertility is unaffected (Bruere and Ellis 1979), and in mice with three trivalents fertility is reduced

by only 25% (Capanna *et al.* 1976). In interracial hybrids of the rodent *Ellobius talpinus* heterozygous for ten fusions, 50% fertility remains, and much of the loss may be related to complications arising from the pairing of the short arms of the telocentrics rather than from unbalanced segregation of the trivalents (Bogdanov *et al.* 1986).

In contrast to this, evidence from a number of organisms indicates that when two or more fusion products with monobrachial homology (that is, when there is homology between only one arm on two metacentrics) are present, the disruptive effects on fertility can be profound. This is presumably the result of difficulties in correctly segregating the multiples which are inevitably produced by meiotic pairing. If two metacentrics with monobrachial homology are present, a chain of four chromosomes consisting of the two metacentrics and the two unfused telocentrics will form, three metacentrics will lead to the formation of a chain-of-five, etc. When more than two metacentrics are present, rings rather than chains may form if all of the arms of the metacentrics have homologues which have also been involved in fusions.

In hybrids of *Mus musculus*, when metacentrics with monobrachial homology are present, fertility is greatly reduced, but in this case the fact that male and female hybrids show different levels of infertility suggests that non-chromosomal hybrid effects may also play a part (Gropp *et al.* 1975, Capanna *et al.* 1976). In hybrids between the species *Rattus sordidus, R. villosissimus* and *R. colletti* however, infertility is almost certainly due to nondisjunction of the resulting multiples (Baverstock *et al.* 1983, 1986). In these three species there has been considerable chromosomal divergence via centric fusion, but genetic divergence is minimal. In an electrophoretic study of 55 loci

in *R. villosissimus* and *R. colletti*, no fixed differences were detected and they differed by a Nei distance of only 0.001. *R. sordidus* showed a fixed difference from the other two species at only one of the 55 loci and a Nei distance from them of 0.08. Even so, in laboratory hybrids among these species, a severe reduction in fertility was observed. On the basis of this evidence, these workers suggested that the reduction in fertility is a direct result of the presence of multiples consisting of four, five and seven chromosomes produced by the various crosses. Assuming their conclusions to be correct, the fact that no natural hybrids between sympatric species of the *Rattus sordidus* complex occur can be explained by invoking selection for premating isolation in order to prevent the production of hybrid individuals, which have lowered fertility levels and hence a lower classical fitness.

The breeding data and observations summarised above, along with data on centric fusions in species complexes of bats and shrews led Baker and Bickham (1986) to propose a model of "speciation by monobrachial fusion". They argued that the minimal meiotic effects of heterozygosity for single fusions allow them to be fixed in isolated populations without the requirement of drastic bottlenecks or founder effects, although these could, of course, accelerate the process. Nevertheless, when hybridisation occurs between two populations in which one or more different fusions have been fixed, "the same centric fusions that became fixed with minimal meiotic problems produce reproductive isolation through a greater level of meiotic impairment" (Baker and Bickham 1986).

This elegant model accounts for the existence of species complexes in which the various species are characterised by the possession of different chromosomal fusions, and it is consistent with the observations of Baverstock *et al.* (1983, 1986) that, although the

species of the *Rattus sordidus* complex show extremely low levels of genetic differentiation, they appear to be well advanced in the speciation process.

While not wishing to detract from the value and general predictiveness of their model, it is necessary for later discussion to deal with one assertion of Baker and Bickham's that is not universally true. Capanna (1982) pointed out that if, in a monobrachial hybrid, a fusion complex segregates correctly, every resulting gamete will be karyotypically identical to the gametes produced by one or other of the parents. Thus further hybridisation or backcrossing will result in a reconstruction of the hybrid condition or a reversion to the parental state. On the basis of this observation, Baker and Bickham stated that whilst other barriers to hybridisation will tend to become less effective under continual hybridisation, the barrier resulting from monobrachial homology will not. On the contrary, Thompson (1956) and Lawrence (1958) have demonstrated that the segregation of multiples is under some degree of genetic control and that the ability to correctly segregate multiples responds to selection. Although their experiments were carried out on translocation multiples, there is no reason to believe that this does not apply equally to fusion multiples. Indeed, the requirements for the production of balanced gametes from partial translocation multiples are even more strict; not only must balanced disjunction take place, but chiasma must be restricted only to the homologous, distal regions.

In a zone of continual hybridisation between populations carrying different fusions, Baker and Bickham's model would predict that premating isolation would evolve, and result in the evolution of discrete species. This is certainly consistent with genetic theory, but the alternative possibility, that the alternate segregation of multiples may also arise, must be considered. Under these

circumstances, the chromosomal fusions of each parental type would introgress into the other parental population although, of course, this would be limited to the rate of introgression of those genes which control multiple segregation. Consequently, a population polymorphic for the different fusions would arise, but unless these were maintained by selection, the polymorphism would be eventually lost. In the presence of recombination however, the daughter population would possess some genetic characteristics of both parental populations, even if the final chromosomal complement resembled only one of the parental forms.

From the above discussion, it is clear that when two populations carrying different fusions meet and encounter low levels of hybrid fertility due to monobrachial homology, premating isolation is not the only evolutionary option open to them. Rather, selection will favour individuals which are able to segregate fusion multiples *and* individuals that mate assortatively. The success of one adaptive strategy negates selection for the other, however, and so an "evolutionary race" between the two strategies will ensue. In this race the chance of evolving the two strategies will not be equal because the existence of a degree of premating isolation will result in the production of fewer hybrids. In turn, these hybrids will have a reduced chance of successfully mating, even if they possess the ability to segregate their chains at meiosis, and so the premating isolation strategy has a headstart. Consequently, if the probability of these two strategies arising is equal, in the majority of cases premating isolation will result. If, however, the hybrids have a higher classical fitness caused by heterosis or some related phenomenon, there is the possibility of a chain-carrying hybrid swarm being established.

6.2 TRANSPOSABLE ELEMENTS AND THE ORIGIN OF CHROMOSOMAL FUSION IN *D. CANCERIDES*

On the basis of the data presented in Chapter 5, it appears that hybridisation has played a role in the evolution of certain of the meiotic configurations observed in *D. cancerides*. However, this most probably occurred after the fixation of fusions in the parental races, and so the two phenomena will be discussed independently.

As Baker and Bickham (1986) pointed out, centric fusion is more commonly encountered in some groups than in others, and in the bats and rodents it is by far the commonest kind of chromosomal rearrangement reported. Given the minimal meiotic difficulties caused by these rearrangements, it is likely that the high frequencies observed in these groups are related to an increased production of fusions rather than a greater rate of fixation.

A similar argument can be applied to the situation in spiders which was discussed in section 1.4.1. In the eight species where fusion of sex chromosomes, autosomes or both has continued to completion, there is no evidence that fixation may have been enhanced by peculiar breeding systems or founder effects.

In *D. cancerides*, the electrophoretic evidence presented in section 5.2 indicates that despite their social behaviour, colony members are not all closely related kin. Consequently, it is probable that *D. cancerides* is not highly inbred. This assumption is also supported by two facts:

(i) populations of *D. cancerides* show little regional variation in alleles or allele frequencies, and

(ii) *D. cancerides* is considerably more polymorphic than other social spiders that are known to routinely inbreed (Lubin and Crozier 1985, Smith 1986).

No convincing models have been put forward to explain the mechanisms behind the origin of the multiple fusions of spiders or the other organisms described above, and given the lack of relevant data, any discussion of these must be purely speculative. If any future research is to be carried out into this phenomenon, it would be most profitably spent investigating the possibility that transposable elements have been responsible.

The "P factors" of *Drosophila melanogaster* are a family of transposable elements which, when activated, cause high frequencies of spontaneous chromosomal breakage and rearrangement, mainly in the germline (Woodruff and Thompson 1980, Engels 1983, Engels and Preston 1984). Activity of P factors is determined by the genetic and cytoplasmic background into which the P factors are placed (Engels 1983). In "P strains" of *D. melanogaster*, P factors are present but generally inactive, but when a P strain is crossed with an "M strain", which does not possess P factors, activity is induced. P factor activity is greater when the P factor donor is male than when the reciprocal cross is formed (Engels 1983). Induction of P factor activity causes spontaneous multiple chromosome breakages and rearrangements in the germline of these hybrids, and often, breakage "hotspots" can be identified (Engels and Preston 1981). These are positions on chromosomes where breakages occur independently in a number of individuals, and are presumably the specific sites where P factors are present. In one case, mutator activity which is presumably P factor mediated, has continued to occur for more than 25 years after the initial hybridisation (Woodruff and Thompson 1980).

Transposon-induced chromosomal rearrangements of the kind found in *D. melanogaster* hybrids have not been demonstrated in any other animal species. However, there are examples of spontaneous rearrangements occurring in other groups, particularly grasshoppers,

after hybridisation, which may well have been induced by a similar process (King 1982). Shaw *et al.* (1983) reported the occurrence of spontaneous chromosomal rearrangements in interracial hybrids of the grasshopper species *Caledia captiva*. 12% of laboratory bred backcross progeny possessed one or more novel rearrangements including Robertsonian fusions and inversions, and fusions were also observed in natural hybrids from the hybrid zone between these two races. Moreover, apparently identical rearrangements of independent origin, both in the natural and laboratory samples, were observed, which indicates that hotspots for rearrangements may exist. Thus, it is probable that an agent similar to the P factors of *D. melanogaster* may be operating in *C. captiva*. Also of interest is the fact that novel electrophoretically detected alleles have spontaneously arisen in some of the hybrids, presumably as a result of the same process (Shaw, pers. comm.). Similarly, Barton *et al.* 1983) suggested that an increase in the frequency of rare alleles in a chromosomal hybrid zone of the grasshopper *Podisma pedestris* may have resulted from an increase in mutation rate following hybridisation.

On the basis of these observations, it is suggested that a similar process may have led to the origin of the fusion races in *D. cancerides*, and also in the other spider species discussed in section 1.4.1. One possible model to explain how this may have occurred follows.

In section 3.1.1 it was mentioned that *D. cancerides* preferentially avoids *Eucalyptus* species. From pollen analyses, it is evident that eucalypts did not become the dominant forest species in southern Australia until as recently as 5000 years ago (Singh *et al.* 1981). Furthermore, in samples from Kangaroo Island it has been demonstrated that before the appearance of eucalypts, *Casuarina*, which is a preferred host tree of *D. cancerides*, was the dominant forest

species (Singh *et al.* 1981). Thus it is reasonable to assume that *D. cancerides* colonised Australia when tree types such as *Casuarina* were more dense and widespread. With the ascendancy of eucalypt species, there was a resultant fragmentation of the *D. cancerides* distribution within the species range. An accompanying reduction in gene flow would have resulted in genetic differentiation of isolated populations and possibly the accumulation or loss of sequences similar to P factors in some populations. Subsequent increases in range, perhaps caused by adaptation to life in eucalypts when necessary, may have led to hybridisation with other isolated populations and the subsequent activation of the mutation process. If the hypothesised P factor analogues preferentially inserted into sequences common to the centromeres, fusion would have been the most common type of chromosomal rearrangement, and would have continued to occur, either rapidly or over a number of generations, until no free telocentrics remained. Over many generations, an inability to segregate chains produced by monobrachial homology would have resulted in stabilising selection finally resulting in populations homozygous for fusions, or perhaps possessing a single sex-linked trivalent as in the CIII cytotype. This set the stage for subsequent, repeated hybridisation along zones of contact between populations saturated with fusions, and the selection for the ability to segregate sex-linked chains.

The scenario described above bears a close similarity to the CPF model described in section 4.4.5(c), however the CPF model was constructed on the assumption that all of the races had a similar origin. On the basis of the electrophoretic data presented in Chapter 5, it now seems likely that the CV and CIX races in fact arose as the result of hybridisation, and so it is no longer necessary to assume that the original fusion process was rapid. Rather, it is only necessary to assume that it was *persistent*.

6.3 HYBRIDISATION AND THE ORIGIN OF PERMANENT TRANSLOCATION HETEROZYGOSITY IN *ISOTOMA* AND *OENOTHERA*

Two relevant electrophoretic studies have been carried out on plants which possess permanent translocation heterozygosity. Although these plants carry partial translocations rather than fusions and are monoecious, both studies have relevance to the problem of the origin of complex sex-linked fusion heterozygosity in *D. cancerides*. These data and a model for the origin of permanent translocation heterozygosity in *Oenothera* will be presented in order to define some of the concepts and processes which must be invoked to explain the hybrid origin of complex sex-linked fusion heterozygosity in *D. cancerides*.

In the Australian plant species *Isotoma petraea*, James *et al.* (1983) reported that populations which carry fixed translocation complexes have an average genic heterozygosity level 12.5 times higher than in those populations without fixed translocation heterozygosity. Fixed genic heterozygosity was observed in some populations, but the extent to which this occurred is unclear. James *et al.* (1983) also failed to report the number of chromosomes involved in the translocation complexes of each population analysed, and whether the degree of heterozygosity was correlated with this. However, population sites are given, and from other work on this species (James 1970, Lavery and James 1987) it is possible to determine the number of chromosomes involved in ring formation in ten of the populations used. On the basis of these data there appears to be no correlation between the proportion of the karyotype involved in the multiples and the level of genic heterozygosity, except that the Pigeon Rock population, which is heterozygous for the lowest number of translocations (6), also has the lowest levels of genic heterozygosity among the permanent

translocation heterozygotes. James *et al.* (1983) argued that the electrophoretic data supported the migration of translocation heterozygosity through the species range via hybridisation, and accompanied by selection for the more genically heterozygous plants. The extent to which permanent translocation heterozygosity is the cause of the increased genic heterozygosity levels in this species remains to be clarified.

In an electrophoretic study of 20 loci in ring-carrying and bivalent-forming species of *Oenothera* Levin (1975) and Levy and Levin (1975) concluded that permanent translocation heterozygosity in this genus has largely resulted from hybridisation, but not from selection for high levels of heterozygosity. Indeed, both the inbreeding ring-formers and the outbreeding bivalent-formers showed very low levels of genic variation when compared to other plant species, and heterozygosity levels were only moderate. While some of the chain-forming populations did show low levels of fixed genic heterozygosity even after twenty generations of selfing, this was not consistent. It also was observed that the older ring-forming taxa had higher levels of heterozygosity than the more recently derived ring formers, perhaps as the result of the subsequent accumulation and fixation of novel variants on their complexes, but again, this must surely be a secondary effect and unrelated to the initial origin of translocation heterozygosity.

On the basis of their electrophoretic data, Levy and Levin (1975) proposed that the origin of translocation heterozygosity in *Oenothera* was "the result of selection for increased fertility in areas of hybridisation between taxa differing by several segmental arrangements." They do not discuss this hypothesis in detail, but are presumably referring to situations where translocations have occurred in the two parental populations such that their chromosomes are not

fully homologous. Consequently, random chromosomal reassortment in hybrid meiosis would lead to the production of duplication/deficiency products. Under these circumstances, alternate segregation would be selected. However, unbalanced gametes would also result from crossing-over in differential segments of chromosomes in hybrids with translocation heterozygosity. In this case, it would be necessary for alternate segregation to occur, *and* for chiasmata to form only in positions distal to regions of non-homology.

If Levy and Levin's interpretation of their data is correct then, in *Oenothera*, the race between the acquisition of premating isolation and correct segregation strategies discussed above has been won by the latter. This outcome would be expected to be more common in flowering plants than in internally fertilised animals because, in plants, true premating isolation would be more difficult to attain. Once the pollen has been removed from an anther, either by an animal vector or air movement, the plant has no direct control over its final resting place. In contrast, animals have a real mate choice, and selection will favour those that choose the mates which will be most likely to produce fertile offspring. The only selective strategies available to a plant to produce a similar effect are either to alter flowering time so that it does not coincide in the two forms (allochrony), or to utilise a different pollination vector. However, in outbreeding plants such as the bivalent-forming *Oenothera* species, both of these strategies carry disadvantages, since if an individual plant has either of these parameters altered then fewer plants will be available for it to fertilise or be fertilised by.

The acquisition of "postmating isolation" (gametic or zygotic incompatibility) may also be disadvantageous to an outcrossing, monoecious plant. Clearly it would be more desirable for a plant's ovules to be fertilised by pollen of its own type, but a plant whose

pollen can also fertilise the opposite type would have an increased fitness, because even hybrid offspring with a low fertility are better than none at all. Moreover, if only one complex is present in the hybrid, its offspring will be, on average, more fertile, because some backcross and F2 progeny will rebound to the parental states.

If the ability to form distal chiasmata and to alternately segregate chains appeared in some outbreeding hybrid individuals, they would be in danger of losing the relevant genes in their offspring through chromosomal reassortment and dilution in future generations. Thus, once this ability was acquired, inbreeding, which does indeed occur in the ring-forming *Oenothera* species (Cleland 1972), would be favoured. Furthermore, this would not be as deleterious to a hybrid as to the bivalent-forming parental types because, as Levy and Levin (1975) have demonstrated, translocation heterozygosity in *Oenothera* does maintain low levels of heterozygosity, even after many generations of inbreeding. Under normal conditions, some of the progeny resulting from hybrid inbreeding would rebound to the parental forms. If, however, self-incompatibility alleles were present in the homozygous parental forms, as they are in bivalent-forming *Oenothera* species (Cleland 1972), these would automatically prevent the formation of homozygotes by inbreeding hybrids. The initially higher levels of polymorphism (and hence heterozygosity), some of it fixed, may also impart a higher level of fitness to the inbreeding hybrids, and finally result in a population consisting entirely of translocation heterozygotes.

It is not intended to portray the preceding model as the best or only possible explanation for the origin of permanent translocation heterozygosity in *Oenothera*. Rather, it has been constructed to demonstrate two points which may be of general importance in considerations regarding the origin of this unusual chromosomal behaviour:

(i) the pursuit of genic hybridity is not necessarily the only explanation for the origin and presence of permanent translocation heterozygosity, and

(ii) permanent translocation heterozygosity can have adaptive value irrespective of any effect it may have on gene segregation patterns. However, such effects may have some relevance to the maintenance of this chromosomal phenomenon when inbreeding is enforced.

6.4 HYBRIDISATION AND THE ORIGIN OF COMPLEX SEX-LINKED FUSION HETEROZYGOSITY IN *D. CANCERIDES*

As was discussed in section 5.5.3, the high levels of polymorphism in the CV and CIX races of *D. cancerides* are construed as strong evidence for a hybrid origin for these two races. Continued hybridisation along a zone of contact would result in the generation of a population of hybrids and hybrid derivatives possessing alleles from both populations, and perhaps some novel alleles produced by the hybridisation process as in hybrids of *Caledia captiva* and *Podisma pedestris* (section 6.2). Sex-linkage of the aldolase locus also supports a hybrid origin, one parental type contributing the aldolase C allele and the other the B allele. It is possible however, that this may be the result of a secondary loss of the B allele from the X-complex and the C allele from the Y-complex.

Before discussing further the mode of origin of complex sex-linked fusion heterozygosity in *D. cancerides*, it is worthwhile to recall some relevant observations from the preceding chapters:

(i) the wide distribution of the CV and CIX races (Fig. 4.78) suggests that these may possess or have possessed some adaptive superiority over the ancestral forms.

(ii) the chiasmata distributions in these two races (Table 4.5) indicate that this chromosomal system does not restrict appreciable amounts of genetic material to males in the long-term.

(iii) there is no evidence that complex sex-linked fusion heterozygosity in *D. cancerides* maintains higher overall levels of genic heterozygosity in males than in females (Table 5.6).

(iv) the raised heterozygosity levels in the two races, particularly in the CIX race (Table 5.6), can be ascribed to higher polymorphism levels rather than to any property of complex sex-linked fusion heterozygosity *per se*.

(v) the electrophoretic data from single colonies (Table 5.1), the low levels of regional variation in gene frequencies (Table 5.3), and the small genetic distances between mainland populations (Table 5.7) all indicate that inbreeding levels in *D. cancerides* have been low, both in the recent and more distant past.

(vi) the chromosomal reordering in the mainland populations may have occurred relatively recently, since there has been little genetic divergence between the mainland populations (Table 5.7).

In the absence of any gross genetic effects resulting from the possession of complex sex-linked fusion heterozygosity, two possibilities exist for the presence of this chromosomal system in *D. cancerides*:

(i) at some time in the past it did impart some selective advantage to the species, or

(ii) it is a functionless remnant of a past hybridisation event, perhaps one in which hybrids were selected for some unrelated quality.

It is improbable that (i) above is correct. The data on chiasmata distributions presented in section 4.3.2 indicate that the proximal position of chiasmata represents the ancestral state, and that distalisation has occurred more recently. Yet, even with the chiasmata distributions observed at the present time (Table 4.5), there can be little long-term restriction on gene recombination. The possible effects of short-term modification of recombination will be dealt with in the next section, but in discussing the origin of this chromosomal system, only the second possibility will be explored.

The high levels of polymorphism in the CIX and CV races reported in Chapter 5 (Table 5.6) are consistent with multiple hybridisation events. If only a small number of hybrids were initially formed, they would have possessed a small portion of the gene pool of the two parental types, and so the final polymorphism levels in the resulting chain-carrying populations would not be substantially higher than those of the parents. Furthermore, the generation of a chain population from the AA and BB populations described in section 4.4.5(b) would be more likely to be realised by the replacement of the AA males with chain-carrying males than by the *de novo* formation of a chain-carrying race and the subsequent replacement of one or both of the parental races. The AA females need not have been *identical* to the females of the new chain-carrying race since, if the AA females possessed some chromosomes with monobrachial homology to those of the hybrid males which were not involved in the chain complex, these would form free-floating rings in the offspring, which would gradually drift to homozygosity by the action of stochastic processes. Thus, in producing a model to explain the origin of complex sex-linked fusion

heterozygosity in *D. cancerides*, the chromosomes not directly involved in chain formation can be ignored. It was mentioned in Chapters 4 and 5 that one of the chain-carrying races may be parental to the other. Nevertheless, many of the principles involved in deriving such a race via hybridisation will be similar, whether or not the parental type possessed complex sex-linked fusion heterozygosity.

Thus it is proposed that at least one chain-carrying race arose by hybridisation along a zone of contact between the hypothesised parental races (AA and BB). The AA race possessed an X-A fusion, and so a chain of three chromosomes in the male - the minimum chain length possible, given the all-or-nothing rule of chromosome fusion in spiders (section 1.4.1) and the presence of an X-A fusion. The BB parent was homozygous for all of its chromosomal fusions, and possessed one X-X fusion and one free X-chromosome. Alternate segregation of the chromosome multiples in the hybrids was either selected or innate. Hybrids generated at the zone which moved into the AA population repeatedly backcrossed with this parental race, and the AA male cytotype was eventually replaced by the present day AB cytotype. This replacement may have resulted from stochastic processes or because the higher heterozygosity of the hybrids conferred a selective advantage on them.

Attempts to construct a computer model to test this have so far been unsuccessful, owing to complications arising from the different segregation patterns of the A- and B-derived X-chromosomes - the A-derived X-chromosome will always remain linked to the A haplotype even after repeated hybridisation events, while the B-derived X-chromosome may segregate with either parental haplotype in hybrids. Despite these difficulties, it is possible to follow the fate of the various haplotypes and X-chromosomes over a small number of generations. Table 6.1 shows the results of backcrosses between the F1 hybrids pictured

in Figure 4.80 and the two parental races. In this table, a distinction is drawn between the A-derived X-chromosome (ax) and the B-derived X-chromosome (x), because the B-derived X of the AB^{x} hybrid can segregate with either the A- or B-complex at meiosis, while the A-derived X is permanently linked to the A-complex. Unusual genotypes in which the B-derived X-chromosome has segregated with the male A haplotype arise from the backcrosses involving the AB^{x} males, but each genotype shown occurs at only half the frequency of those shown for the other crosses, assuming that all crosses are equally fertile and viable. The backcrosses to the BB race have been included for completeness, but the following discussion concentrates on the backcrosses to the AA race only.

The left hand side of Table 6.1 represents the genotypic patterning on the AA side of the proposed hybrid zone. As hybrid derivatives move further from the centre of the hybrid zone into the AA population, the frequency of parental AA forms in the region will increase, and so the probability of their backcrossing with the AA population will be much greater than the chance of their crossing with each other. It is recognised that hybrid forms resulting from F2 and higher level hybridisations will also be present in this region, and this will be dealt with separately.

Of note in Table 6.1 is the fact that the majority of the offspring belong to the AA parental genotypes and the required AB heterozygote. The next most common genotype is the $A^{ax}B^{x}$ female which, on further backcrossing produces both sexes of the AA phenotype, itself, and AB^{x} males. The AB^{x} males in turn produce both $A^{ax}A$ males and $A^{ax}B$ males, the female F1 genotype, and AA females heterozygous for the two kinds of X-chromosome. Overall, however, this last genotype is comparatively rare, since it is only produced by the AB^{x} backcross, and in subsequent backcrosses only half of the offspring

carry the B-derived X associated with the A haplotype. Thus there is an "$A^{ax}B^{X}/AB^{X}$ cycle" which generates the AA parental genotypes and $A^{ax}B$ males.

The fact that parental AA types and $A^{ax}B$ males are produced at high frequencies by backcrossing with the AA population means that the commonest mating combinations in this area will be $A^{ax}A^{ax}$ X $A^{ax}A$ and $A^{ax}A^{ax}$ X $A^{ax}B$. The frequency of the $A^{ax}A$ males will be equal to the frequency of the $A^{ax}B$ males, but this will be offset somewhat by the immigration of $A^{ax}A$ males from the AA population.

Table 6.2 shows the F2 progeny derived from both reciprocal crosses of the AA and BB populations, and the frequencies of the four male genotypes produced. Of particular significance here is the fact the the two most commonly produced male genotypes are the required $A^{ax}B$ genotype, and the $B^{X}B$ genotype which, on backcrossing produces $A^{ax}B$ males but not AB^{X} or $A^{ax}A$ males (Fig. 4.80). In contrast, the $B^{X}B^{X}$ females which generate the reciprocal backcross, and the less desirable AB^{X} males, are produced at only one third of the frequency on the $B^{X}B$ males in the F2 progeny. When the F2 progeny are backcrossed to the AA population, the AB^{X} males and $A^{ax}B^{X}$ females feed into the $A^{ax}B^{X}/AB^{X}$ cycle, which produces more $A^{ax}A$ males than the required $A^{ax}B$ males, but this is more than compensated for by the excess of $A^{ax}B$ and $A^{ax}B$-producing $B^{X}B$ males from the F2 backcrosses. Thus, when F1 hybrids, first generation backcrosses, F2 hybrids and second generation backcrosses are considered together, the two most frequently generated males genotypes are the parental $A^{ax}A$ males and the required $A^{ax}B$ males. Of these, the $A^{ax}B$ males are produced most frequently.

There is insufficient space to consider the fate of those genotypes which result from the segregation of the B-derived X-chromosome with the A haplotype, but these are of lesser importance,

since the male genotypes appear at a much lower frequency than the $A^{ax}A$ and $A^{ax}B$ male genotypes.

Thus, it appears that, in the earlier hybridisation events at least, the $A^{ax}B$ males may well begin to replace the $A^{ax}A$ males in the localised region of the AA distribution near the hybrid zone, irrespective of any adaptive superiority of the $A^{ax}B$ chromosomal configuration.

If it is assumed that the higher level of polymorphism (and hence heterozygosity) produced by the mixing of the AA and BB gene pools conferred a selective advantage on individuals with a hybrid ancestry, the population which arose in proximity to the hybrid zone would spread further into the region occupied by the AA population. The AA males and females with a hybrid origin, and the $A^{ax}B$ males would be the commonest genotypes, but the other types would occur at lower frequencies. Since none of the other types consistently reconstruct only their own genotype on backcrossing, their frequency would only be maintained in the population by migration from the hybrid zone. However, this would maintain the drive which produces excess $A^{ax}B$ males, finally causing this male genotype to predominate and subsequently replace the $A^{ax}A$ genotype completely. Once this occurred, all individuals in the AA population would have a hybrid ancestry, and so the adaptive advantage of individuals from the hybrid zone would be reduced. Consequently, competition would reduce the introgression rate of genes and chromosomes from the hybrid zone into the new $A^{ax}B/A^{ax}A^{ax}$ population.

In the BB population, genic introgression may occur through the effects of hybrid advantage, but with gene recombination and the absence of an X-A fusion in this population, this could take place without being accompanied by the production of permanent complex sex-linked fusion heterozygosity.

Thus, although it has not been possible to produce a working computer model of this phenomenon, on the basis of the limited analysis above it does appear that complex sex-linked fusion heterozygosity may have arisen in *D. cancerides* as the result of hybridisation. With selection favouring genic hybridity, complex sex-linked fusion heterozygosity was favoured, but only indirectly. If this view is correct, complex sex-linked fusion heterozygosity is indeed merely a functionless remnant of past selection for genic polymorphism and heterozygosity.

6.5 COMPLEX SEX-LINKED FUSION HETEROZYGOSITY AND SOCIAL BEHAVIOUR

On the basis of the discovery of sex-linked translocation heterozygosity in a number of termite species, the possibility that the existence of chromosomal systems of this kind may be causally related to the occurrence of social behaviour has been discussed in some detail (Luykx and Syren 1979, Lacy 1980, 1984, Leinaas 1983, Crozier and Luykx 1985). That a similar chromosomal phenomenon occurs in *D. cancerides*, which also displays a form of social behaviour, adds strength to this correlation (Rowell 1985, 1986). Nevertheless, given the electrophoretic and chromosomal data presented here, the possibility that the presence of these two phenomena in *D. cancerides* is anything but coincidental seems remote.

In the Isoptera and *D. cancerides*, forms exist which do not possess permanent chromosomal heterozygosity, and so in both cases it has clearly arisen after the evolution of social behaviour. Thus, assuming that social behaviour does not affect the incidence of chromosomal rearrangements, two possible causal relationships exist: (i) the evolutionary steps leading to the production of permanent sex-

linked translocation heterozygosity are equally likely to occur in social and non-social species, but it is positively selected for in social species for some adaptive advantage it confers, or (ii) some aspect of social behaviour increases the rate of fixation.

The only aspect of (ii) which is likely to affect the fixation of chromosomal heterozygosity in *D. cancerides* is inbreeding. However, on the basis of the electrophoretic analysis of colonies and the low levels of regional differentiation observed, it appears that inbreeding is not a significant factor.

In section 4.4.6 it was pointed out that the distribution of chiasmata does result in a low degree of linkage between genes in the short term. This effect is minimal however, since in both the CV and CIX races, fewer chromosomes are sex-linked than not. Furthermore, even if sex linkage were higher, the colonies are not kin groups and so complex sex-linked fusion heterozygosity would do little to enhance uniformity, and average relatedness must still be considerably lower than 50%. Thus, neither of the selective models which have been proposed to explain the presence of this chromosomal system in termites - the uniformity model of Luykx (1979) and the haplodiploidy analogue model of Lacy (1981) - is applicable to *D. cancerides*. Therefore it is considered that complex sex-linked fusion heterozygosity arose in *D. cancerides* as a chance event that could equally well have arisen in any non-social species which fulfilled the prerequisites of having populations with different chromosome fusions including one with an X-A fusion, and the selected or innate ability to segregate chromosome chains in such a way as to produce balanced gametes.

6.6 SUMMARY

The possibility was raised that the fusion process in *D. cancerides* may have resulted from the activity of transposable elements similar to the P-factors of *Drosophila melanogaster*. The origin of complex sex-linked fusion heterozygosity in *D. cancerides* was discussed in the light of the "speciation by monobrachial homology" model of Baker and Bickham (1986). It appears that the evolution of this chromosomal system has been facilitated by the innate or selected ability of this species to segregate fusion multiples alternately. The presence of complex sex-linked fusion heterozygosity may well be the result of selection for genic heterozygosity rather than for any adaptive value of structural heterozygosity *per se*. Neither the haploidiploid analogue nor the uniformity model, which have been used to explain the presence of complex sex-linked translocation heterozygosity in termites, is applicable to *D. cancerides*. The fact that this species displays both social behaviour and complex sex-linked fusion heterozygosity is considered to be purely coincidental.

BACKCROSS TO AA POPULATION	BACKCROSS TO BB POPULATION
$A^{ax}A^{ax}$ by $A^{ax}B$	**$B^{x}B^{x}$ by $A^{ax}B$**
$A^{ax}A^{ax}$, **$A^{ax}B$**, **$A^{ax}A^{ax}$**, **$A^{ax}B$**	$A^{ax}B^{x}$, $B^{x}B$, $A^{ax}B^{x}$, $B^{x}B$
$A^{ax}A^{ax}$ by AB^{x}	**$B^{x}B^{x}$ by AB^{x}**
$A^{ax}A$, $A^{ax}B^{x}$, **$A^{ax}A$**, $A^{ax}B^{x}$	AB^{x}, $B^{x}B^{x}$, AB^{x}, $B^{x}B^{x}$
$A^{ax}A^{x}$, **$A^{ax}B$**, $A^{ax}A^{x}$, **$A^{ax}B$**	$A^{x}B^{x}$, $A^{x}B^{x}$, $B^{x}B$, $B^{x}B$
$A^{ax}B^{x}$ by $A^{ax}A$	**$A^{ax}B^{x}$ by $B^{x}B$**
$A^{ax}A^{ax}$, **$A^{ax}A$**, $A^{ax}B^{x}$, AB^{x}	$A^{ax}B^{x}$, **$A^{ax}B$**, $B^{x}B^{x}$, $B^{x}B$
$A^{ax}B^{x}$ by $A^{ax}A$	**$A^{ax}B^{x}$ by $B^{x}B$**
$A^{ax}A^{ax}$, **$A^{ax}A$**, $A^{ax}B^{x}$, AB^{x}	$A^{ax}B^{x}$, $A^{ax}B$, $B^{x}B^{x}$, $B^{x}B$

Table 6.1 Karyotypes of progeny from backcrossing of the F1 hybrids of both AA x BB reciprocal crosses to the parental types. The notation of Figure 4.80 is used, except a distinction is drawn between the A-derived X-chromosome (ax) and the B-derived X (x). Progeny that reconstruct the AA parental karyotype and the $A^{ax}B$ karyotypes are bolded. The bottom line of the AB^{x} crosses are the result of the B-type X-chromosome segregating with the A haplotype. The karyotypes presented for these crosses do not imply that twice as many offspring are produced in these cases. Two points are worthy of note: (i) in the $A^{ax}A^{ax}$ by $A^{ax}B$ backcross, the parental types are reproduced and no other karyotypic patterns occur, and (ii) the total number of $A^{ax}A$ and $A^{ax}B$ males produced in the backcrosses to the AA population are equal, and both are more common than the third type of male, AB^{x}.

$A^{ax}{}_{B} \times A^{ax}{}_{B}{}^{x}$

$A^{ax}{}_{A}{}^{ax}$, $A^{ax}{}_{B}{}^{x}$, $A^{ax}{}_{B}$, $B^{x}{}_{B}$

$AB^{x} \times A^{ax}{}_{B}{}^{x}$

$A^{ax}{}_{A}$, AB^{x}, $A^{ax}{}_{B}{}^{x}$, $B^{x}{}_{B}{}^{x}$

$A^{ax}{}_{A}{}^{x}$, $A^{x}{}_{B}{}^{x}$, $A^{ax}{}_{B}$, $B^{x}{}_{B}$

MALE GENOTYPE FREQUENCIES

$A^{ax}{}_{A}$	$A^{ax}{}_{B}$	$B^{x}{}_{B}$	AB^{x}
1	3	3	1

Table 6.2 F2 genotypes after initial hybridisation between the hypothesised AA and BB parental populations, and the relative frequencies of the four male genotypes generated. The cross involving the AB^{x} genotype produces extra genotypes resulting from the segregation of the B-derived X-chromosome with the A haplotype, but the types shown for this cross will occur at only 50% of the frequency of those shown for the other cross if it is assumed that both crosses are equally fertile.

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APPENDIX 1.

Electrophoretic data for sparassid genera. Genotypes are coded in the following order : GP1, GP2, GP3, HK, AK, FUM, LDH, GAPDH, GPD, MDH1, MDH2, PGD, Gd, ALD, GPI, PGM, AAT1, AAT2.

tII (Queensland)

males
AAAAAABBBBCCAAAACCBBBBCCBBBBFFEGCCBBBBBB
AAAAAABBBBCDAAAACCBBBBCCBBBBFFEECCBBBBBB
AAAAAABBBBCCAAAACCBBBB BDFFEECCBBBBBB
AAAAAABBBBCDAAAACCBBBB BBFFEECCBBBE
AAAAAABBBBCCAAAACCBBBB BBFFEECCBBBB
AAAAAABBBBDDAAAACCBBBBCCBBBBFFEECCBBBEBB

females
AAAAAABBBBCCAAAACCBBBB BBFGEECFBBBB
AAAAAABBBBCCAAAACCBBBB BBFFEECFBBBB
AAAAAABBBBCCAAAACCBBBB BBCFEECCBBBB
AAAAAABBBBCCAAAACCBBBB BBFFEECFBBBBBB
AAAAAABBBBACAAAACCBBBBCCBBBBFFEECCBBBBBB
AAAAAABBBBCCAAAACCBBBB BBFFEECCBBBB
AAAAAABBBBDDAAAACCBBBBCCBBBBFFEECCBBBBBB
AAAAAABBBBCCAAAACCBBBB BBFFEECCBBBB
AAAAAABBBBCCAAAACCBBBB BBFGEECCBBBBAB
AAAAAABBBBCCAAAACCBBBB BBFGEECFBBBB
AAAAAABBBBCCAAAACCBBBB BBFFEECCBBBB

juveniles
AAAAAABBBBCCAAAACCABBBCCCDBBFFEECCBBEFBB
AAAAAABBBBCCAAAACCBBBBCCBCBBFFEECCBBEFBB
AAAAAABBBBCCAAAACCABBBCCBBBBFFEECCBBBBBB

tII (Victoria)

males
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEFCC EEBB
AAAAAABBBBCCAAAACCBBBBCCAACEFFEECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBB FFEEBBBBEE
AAAAAABBBBCCAAAACCBBBB CCDFEECCBBEEBB
AAAAAABBBBCCAAAACCBBBB CCFFEEBCBBEE
AAAAAABBBBCCAAAACCBBBB CCFFCEBCBBEE
AAAAAABBBBCCAAAACCBBBB CCFFCCBBBBEE
AAAAAABBBBCCAAAACCBBBB CCEFEECCBBEE
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEGBCBBEEBB

females
AAAAAABBBBCCAAAACCBBBBCCBBCEFFEECCBBBEBB
AAAAAABBBBCCAAAACCBBBB CCFFEE BBEEBB
AAAAAABBBBCCAAAABCBBBBCCBBCCFFEGCCBBEEBB
AAAAAABBBBCCAAAACCBBBB CCCFEHBCBBEE

juveniles
AAAAAABBBBCCAAAACCBBBB CCFFEECCBBEEBB

mII (South Australia)

males
AAAAAABBBBCCAAAACCBBBBBCBBCEFFEECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBDEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEEBCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBBBCFEECEBBEEBB
AAAAAABBBBCCAAAABCABBBCCBBBCCFAECEBBEEBB
AAAAAABBBBCCAAAABCABBBCCBBCCFFEGCCBBEEBB

females
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFCECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFGGCCBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBB
AAAAAABBBBCCAAAACCABBBCCBBBBFFEECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBDEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAABBBBCCAAAACCABBBCCBBBBFFEECCBBEEBB

mII (Victoria)

males
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAAABBBCCAAAACCBBBBCCBBCCFFEECCBBCCBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFGEECCBBDEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCEECECCBBEEBB
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AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFIEECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBBBCFEECCBBEEBB

females
AAAAAAABBBBCCAAAACCABBBCCBBCCFFEECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEGCCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBDEBB
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AAAAAAABBBBCCAAAACCBBBBCCBBBCFICECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBBCFFEEBCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCDFFEECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBBCFFEECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFIEEBCBBEHBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFIEECCBBEHBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEGCCBBCEBB

mII (Kangaroo Island)

females
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB

males
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBB FFEECCBBEEBB

CIII (Perth)

females
AAAAAAABBBBCCAAAABCBBBBCCBBBBFFEICCBBEEBB
AAAAAAABBBBCCAAAACCBBBDCCBBBBFFEICCBBEEBB
AAAAAAABBBBCCAAAABCBBBBCCBBBBFFEECCBBEEBB
AAAAAAABBBBCCAAAABCBBBBCCBBBBFFEECCBBEEBB
AAAAAAABBBBCCAAAABCBBBBCCBBBBFFEICCBBEEBB

juveniles
AAAAAAABBBBCCAAAACCBBBBCCBBADFFEICCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBADFFEICCBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBADFFEECCBBEEBB

CV

males
AAAAAAABBBBCCAAAACC   CCBBBCFFEECCBBEEBB
AAAAAAABBBBACAAAACCBBBBCCBBBCFFEEBBBBEEBB
AAAAAAABBBCCAAAACC   CCBBBCFFEEBBBBEEBB
AAAAAAABBBCCAAAACCBBBBCCBBBCFFEEBBBBEEBB
AAAAAAABBBCCAAAACC   CCBBCCFFEEBCBBEEBB
AAAAAAABBBCCAAAACC   CCBBBBFFEECCBBDDBB
AAAAAAABBB AAAACC   CCBBBCFFEEBBABDEBB
AAAAAAABBBCCAAAACC   CCBBBBFFDDBCBBEGBB
AAAAAAABBB AAAACC   CCBBBCFFDEBCBBEEBB
AAAAAAABBBCCAAAACC   CCBBBCFFEECCBBDEBB
AAAAAAABBBCCAAAACCBBBBCCBBBBFFEECCBBEEBB
AAAAAAABBBCCAAAACC   CCBBBCFFEEBCBBDEBB
AAAAAAABBBCCAAAACCBBBBCCBBBBFHDEBCBBDEBB
AAAAAAABBBCCAAAACCBBBBCCBBBCFFDDBBBBEEBB
AAAAAAABCBBCCAAAACCBBBBCCBBBBFFEEBCBBEEBB
AAAAAAABBBB AAAACC   CCBBBBFFEEBBBBDEBB
AAAAAAABBBB AAAACC   CCBBBCFFEEBBBBDEBB
AAAAAAABBBCCAAAACCBBBBCCBBBCFFEEBBBBEEBB
AAAAAAABBBCCAAAACC   CCBBBBFFEEBBBBEEBB
AAAAAAABBABCCAAAACC   CCBBBCFFEEBBBBEEBB
AAAAAAABBBCCAAAACCBBBBCCBBBBFFEEBCBBEEBB
AAAAAAABBBCCAAAACCBDBBCCBBBCFFEEBBBBEEBB
AAAAAAABBBCCAAAACCBBBBDDBCCCFIEEBCBBEEBB

females
AAAAAAABBABCCAAAACCBBBBCCBBCCFFDEBCBBEEBB
AAAAAAABBBCCAAAACCBBBBCCBBCCFIEECCBBEEBB
AAAAAAABBBCCAAAACC   CCBBCCFFEECCBBEEBB
AAAAAAABBBCCAAAACC   CCBBCCFFEEBCBBEEBB
AAAAAAABBBCCAAAACC   CCBBCCFFEEBCBBEEBB
AAAAAAABBBCCAAAACC   CCBBBBFFEEBBBBEEBB
AAAAAAABBB AAAACC   CCBBBBFFEEBBBBBEBB
AAAAAAABBB AAAACC   CCBBBCFFEEBBBBEEBB
AAAAAAABBBCCAAAACC   CCBBBBFFEECCBBEEBB
AAAAAAABBBCCAAAACCBBBBCCBB FFEEBBBBDEBB
AAAAAAABBB AAAACC   CCBBBBFFEEBBBBEEBB
AAAAAAABBBCCAAAACCBBBBCCBBBBFJEECCBBEEBB
AAAAAAABBBCCAAAACDBBBBCCBBBBFFEHCCBBEEBB
AAAAAAABBBCCAAAACCBBBBCCBBBBFFEECCBBEGBB
AAAAAAABBBCCAAAACC   CCBBBBFFEEBCBBBEBB
AAAAAAABBBCCAAAACC   CCBBCCFFEEBBBBDDBB
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AAAAAAABBBBCCAAAACCBBBBCCBBBCFFEECCBBEEBB
AAAAAAABCBBCCAAAACC   CCBBBCFIEEBBBBEEBB
AAAAAAABBBBCCAAAACC   CCBBBCFFEEBBBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBCCCFEEBBBBEEBB
AAAAAAABBBBCCAAAACC   FFEEBBBBBBBB
AAAAAAABBBBACAAAACC   CCBBBCFFEEBBBBEEBB
AAAAAAABBBBCCAAAACCBBBBCCBBBCFFEEBBBBEEBB
AAAAAAABBB   AACC   BCFFEE   EEEE
AAAAAAABBB   AACC   CCFFEE   EEEE

juveniles
AAAAAAABBB AAAACC   CCBBBBFFEECCBBEGBB

CANBERRA HYBRIDS

males
AAAAAABBBBCCAAAACCBDBBCCBBCCFFEECCBBEEBB
AAAAAABBBBCCAAAACCBBBECCBBBCCFEECC EEBB
AAAAAABBBBCDAAAACCBBBBCCBBBCFFEEBC EEBB
AAAAAABBBBCCAAAACCBDBBCEBBBBFFEECC EEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCDEDEBC DEBB
AAAAAABBBBCCAAAACCBBBBCEBBCCFFEEBB EEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFEECC EEBB

females
AAAAAABBBBCCAAAACCBBBBCCBBCCCFEECC EEBB
AAAAAABBBBBBAAAACDBBBBCCBBCCFFEECC EEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEEBCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCCFEECCBBEEBB
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AAAAAABBBBCCABAACCBBBBCEBBCCFIEEBCBB BB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEHBCBB BB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFEHBCBB BB

CIX

males
AAAAAABBBBCCABAACCABBBCCBBBCFFCECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCEBBBCFFDEBCBBEEBB
AAAAAABBBBCCAAAACCBBBBCEBBBCFFCEBC EEBB
AAAAAABBBBCCABAACCBBBBCCBBBCFFCECC EEBB
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AAAAAABBBBCCAAAACCBBBBCCBBBCCFACBCBBDDBB
AAAAAABBBBCCAAAACCBBBBCCBBBBCFBECC EEBB
AAAAAABBBBCCAAAACCBBBBCEBBBCFFCEBC EEBB
AAAAAABBBBCCABAACCBBBBCCBBBCFFBEBC EEBB
AAAAAABBBBCCAAAACCBBBBCCBBBBFFEECCBBEEBB
AAAAAABBBBACAAAACCBBBBCCBBBCFFEEBBBBEEBB
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AAAAAABBBBCCAAAACCBBBBCCBBBBFFEHBBBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFJEHBBBBDEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFEEBC EEBB
AAAAAABBBBCCAAAACCBDBBCCBBBCFFEEBB EEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFCEBCBBAABB
AAAAAABBBBCCAAAACCABBBCCBBBCFFEEBCBBEEBB
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AAAAAABBBBCCABAACCBBBBCCBBBCFFEECC EEBB
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AAAAAABBBBCCAAAACCBBBBCCBBBCFFEEBC DEBB
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AAAAAABBBBCCAAAACCBBBBCCBBBBFFEECC EEBB
AAAAAABBBBCCAAAACCBBBBCCBBBBCFEECC EEBB
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AAAAAABBBBCCAAAACCBBBBCCBBBCFFEECC EEBB
AAAAAABCBBCCAAAABCBBBBCDBBBCFFCEBBBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFEEBB EEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFEEBC EEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFEEBCBBEEBB
AAAAAABBBBBBCAAAACCBBBBCCBBBCFFEEBBBBEE
AAAAAABBBBCCAAAACCBBBBCCBBBBFFEEBBBBEEBB
AAAAAABBBBCCAAAACCBBBBCEBBBCFFEHABBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFEEBCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFEECCBBEEBB

females
AAAAAABBBBCCAAAACCBBBBCEBBCEFFBEACBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFCEBCBBDEBB
AAAAAABBBBCCAAAACDBBBBCCBBCCFFCEBCBBDDBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFIEEBBBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFACBCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEEBBBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCAFAEBBBBEEBB
AAAAAABBBBCCAAAACDBBBBCCBBCCFFCEBCBBEEBB
AAAAAABBBBCCABAACCBBBBCEBBCCFFEEBBBBEEBB
AAAAAABBBBCCAAAACCBBBBCEBBCCFFEEBCBBEEBB
AAAAAABBBBCCABAACCBBBBCCBBCCFFEEACBBEEBB

AAAAAABBBBACAAAABBBBBBCCBBCCAFCEBCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFCECCBBEEBB
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AAAAAABCBBCCAAAACCBBBBCCBBBBJJEEBBBBBDEBB
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AAAAAABBBBCCAAAACCBBBBCCBCCCFFEEBCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCFFBECCBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEEBCBBEGBB
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AAAAAABBBBCCAAAACCBBBBCCBBBCFIEEBCBBEEBB
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AAAAAABBBBCCAAAACCBBBBCCBBCCCFEEBC EEBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEEBC EEBB
AAAAAABBBBCCAAAACCABABCCBBBBFFEEBCBBEEBB
AAAAAABBBBAAAAAACCCCBBCCBBCCCFEEBBBBEEBB
AAAAAABBBBCCAAAACC BBCCBBCCFFEEBBBBEEBB
AAAAAABBBBCCAAAACCBBBBCCBBBCEFCEBCBBEEBB
AAAAAABBBBCCABAAACBBBBCCBBCCFGEEBC EEBB
AAAAAABBBBCCAAAACCBBBBEEBBCCFFEECDBBEEBB
AAAAAABBBBCCAAAACCBDBBEEBCCDFFEECCBBEGBB
AAAAAABBBBCCAAAACCBBBBCCBBCCFFEECCBBEEBB

tII (Tasmania)
AAAAAABBBBCCAAAADDBBBBCDBDBBBBEEBCBBEEAA

APPENDIX 2.
Electrophoretic data for generic comparisons. Genotypes are coded in the following order : GP1, GP2, GP3, HK, AK, FUM, LDH, GAPDH, GPD, MDH1, MDH2, PGD, Gd, ALD, GPI, PGM, IDH1, 1DH2, AAT1, AAT2.

	SELENOPS
S1	AABBAADD AABBAAAAAAACBBAAEEAAEEDDCC
S2	AAAAAAEE AABBAAAABBBBBBDDDDFFAADDAA
S3	AAAAAAEE AABBAAAABBBBBBCCDDAAAADDAA
S6	AAAAAAEE AABBAAAABBBBBBBBDDAAAADDAA
S8	AAAAAAEEAAAABBAAAABBBBBBDDCCAAABDDAA
	DELENA
DWJ2	CCAAAACCBBBBCCAAAABBBBAAGGDDDDGGEGBB
	ISOPODA
I31	BBAAAACCBBBBCCAADDBBBBCCCCEEGGEEEEBB
I107	BBAAAACCBBBBCCAADDCDBBCCCCEEGGEEEEBB
I130	BBAAAACCBBBBCCAADDEEBBCCCCEECCEEEEBB
I150	BBAAAACCBBBBCCAADDDDBBCCCCEEGGEEEEBB
I18	BBAAAACCBBBBCCAABBBBBBEEFFEEEDDBBBB
I29	BBAAAACCBBBBCCAADDFFBBDDCCEEEEFFEEBB
I87	BBAAAACCBBBBCCAADDFFBBDDCCEEEEFFEEBB
I88	BBAAAACCBBBBCCAADDFFBBDDDEEEEFFEEBB
	OLIOS
O8	DDAAAABBBBBBCCAACCBBBBEEEEEEEEFFCDBB
O14	DDAAAABBBBBBCCAACCBBBBEE EEEEFFCDBB
O16	CCAAAABBBBBBCCAACCBBBBEEEEEEEEEFCDBB
O23	DDAAAABBBBBBCCAACCBBBBEEEEEEEEFFBDBB
	HETEROPODA
H1	BBAAAACCBBAAAAAAAAFFBBBBEEAABBFFEEBB
H2	BBAAAACCBBAAAAAAAACFBBFF DDFFCCBBBB
H4	BBAAAABBBBAAAAAAAaBBCCBBBBBBEEAABB

Chromosoma (Berl) (1985) 93:169–176

CHROMOSOMA

Complex sex-linked fusion heterozygosity in the Australian huntsman spider *Delena cancerides* (Araneae: Sparassidae)

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Abstract. In the vast majority of spider species studied to date, the karyotype is homogeneous in morphology and exclusively telocentric. The sex-determining system consists of one to three X chromosomes in the male and, correspondingly, two to six in the female. This is the case in species of huntsman spiders belonging to the genera *Heteropoda* (2n = 38 + 3X), *Isopoda, Olios,* and *Pediana* (2n = 40 + 3X) and some populations of the colonial species *Delena cancerides* (2n = 40 + 3X). In other populations of *D. cancerides*, wholesale fusion of the karyotype has occurred, reducing the standard huntsman karyotype of 43 telocentric chromosomes to 21 metacentrics and 1 telocentric. Eight of the centric fusion products, including an X-autosome fusion, are maintained in the heterozygous condition in males and, with the single telocentric, form a chain of nine chromosomes at meiosis. The two complexes comprising the chain behave as neo-X and neo-Y chromosomes, and thus the ancestral $X_1X_2X_3$♂:$X_1X_1X_2X_2X_3X_3$♀ sex-determining system has been converted to a system of six X and four Y chromosomes in the male and twelve X chromosomes in the female. Since sex-linked complex heterozygosity is also found in a number of species of social termites, it is suggested that such heterozygosity may have adaptive significance for a colonial lifestyle. Breakdown products of the chain of nine are present in specimens of *D. cancerides* from Canberra and these appear to represent hybrid products between the 2n = 22 and 2n = 43 forms. Hybridisation may also have been involved in the origin of the chain-forming races.

Introduction

Permanent translocation heterozygosity, defined by the regular presence of ring or chain multiples at meiosis, is well known in plants. Here, two rather different systems have been described. In *Oenothera, Rhoeo*, and *Isotoma* all individuals are structural heterozygotes regardless of sex. However, in some populations, different-sized rings or chains may be present. In *Oenothera* and *Isotoma* the system is characterized by a high degree of inbreeding or autogamy ("selfing"), regular alternate chromosome segregation and amongst other things, a system of balanced lethals which act either in the gametes or in the zygote (Cleland 1972; James 1965, 1970). The second system is found in the mistletoe genus *Viscum*. Here only male plants consistently carry multiples, although in a few cases small rings may be present in some females within a population (Wiens and Barlow 1979). While this system is clearly related to the occurrence of dioecy, its relationship to inbreeding has not been clarified.

In animals, permanent translocation heterozygosity of the type found in *Oenothera* and *Isotoma* is not known. One reason for its absence may be that animal populations, due to their greater mobility, rarely experience levels of inbreeding equivalent to those that commonly occur in plants. Thus, to parallel the phenomenon of autogamy would require hermaphroditism which is much rarer in animals than in plants.

On the other hand, the common occurrence of sex chromosomes in animals and the possibility of X-autosome rearrangements lends itself to a form of translocation heterozygosity which, like that of *Viscum,* manifests itself in one sex only. Such a mechanism may explain the chains of nine chromosomes observed at meiosis in the centipede *Otocryptops sexspinosus* (Ogawa 1954) and the fixed trivalent observed in the males of some populations of the jumping spider *Pellenes* (Maddison 1982). A system comparable to that found in *Viscum* is well known in the Isoptera (termites). Here, *Kalotermes approximatus* (Syren and Luykx 1981) and *Incisitermes schwarzi* (Syren and Luykx 1977) regularly carry chain multiples of 11 to 17 chromosomes. While no sex chromosomes are evident in these species, as is also the case in *Viscum*, there is good reason to assume that the chains observed in termites were initiated by a rearrangement event involving an original X chromosome and an autosome. Further translocations have resulted in what, from a segregational point of view, is effectively a multiple XY sex-determining mechanism.

A system which has not been adequately resolved is that found in the monotremes (Murtagh 1977). Here, while multiple chains involving both autosomes and sex chromosomes occur in males, the chromosomal behaviour in the female remains to be clarified.

The present paper deals with a case of sex-linked translocation heterozygosity in the common Australian huntsman spider, *Delena cancerides*, which is similar in many respects to that described in termites.

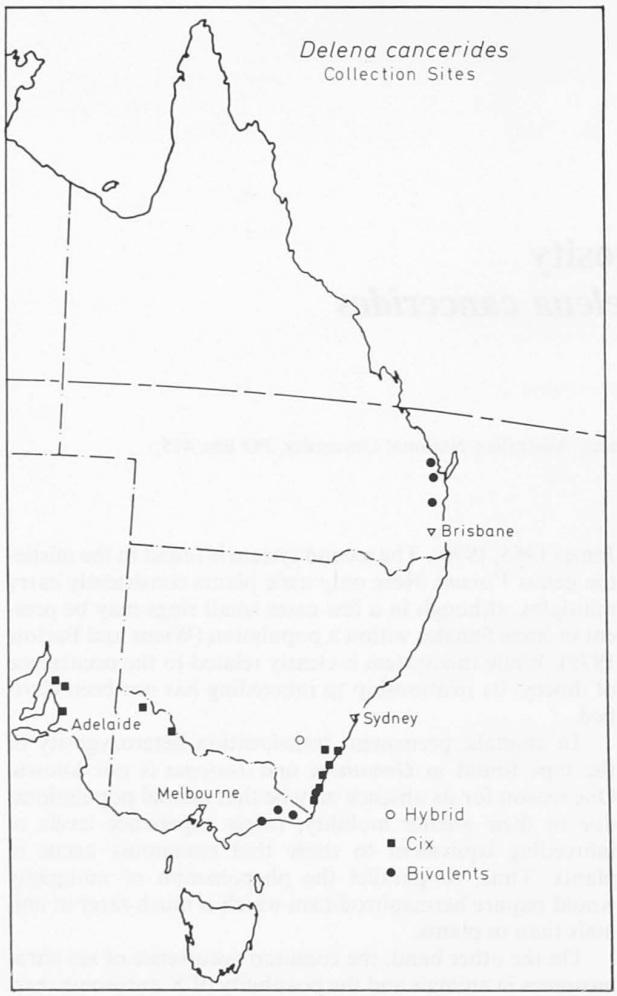


Fig. 1. Collection sites for *Delena cancerides* in S.E. Australia. This map shows the location of populations with bivalent formation and chain formation at meiosis and the single hybrid population from Canberra

Materials and methods

Background data. Sparassids (huntsman spiders) have a worldwide distribution and are represented in Australia by approximately 200 described species, mainly from the genera *Olios, Isopoda, Heteropoda* and *Pediana*. Huntsmen usually live under bark or in foliage, and capture prey at night by stalking rather than using a snare or orb web. They are opportunistic predators eating any arthropods, frogs or small reptiles encountered.

Huntsmen are usually solitary, although male-female pairs are sometimes encountered (personal observations). The females guard their egg sac and tolerate the presence of young for a short period after hatching. The newly hatched young may be fed by the female from a droplet exuded from the mouth. After 2 to 3 weeks the mother's 'tolerance phase' ends and young that have not dispersed are eaten.

A notable exception is the endemic and widespread species *Delena cancerides* which is found throughout mainland

Table 1. Karyotypic data for the five huntsman genera examined

Species	Number of individuals examined	Mitotic chromosome counts ♂	♀	Total arm count (♂)	Male meiosis
Pediana regia	42	43t	46t	43	$20^{II}+3X$
Olios spp.	4	43t	46t	43	$20^{II}+3X$
Isopoda spp.	34	43t	46t	43	$20^{II}+3X$
Heteropoda procera	1	41t	–	41	$19^{II}+3X$
Heteropoda sp. nov	1	41t	–	41	$19^{II}+3X$
Delena cancerides	29	21m+ 1t	–	43	$C^{IX}+6^{II}+X$
Delena cancerides	10	43t	–	43	$20^{II}+3X$
Delena cancerides	1	19m+ 5t	–	43	$2^{CV}+1C^{III}+5^{II}+X$
Delena cancerides	1	9m+25t	–	43	$C^{IX}+12^{II}+X$
Delena cancerides	1	20m+ 3t	–	43	–

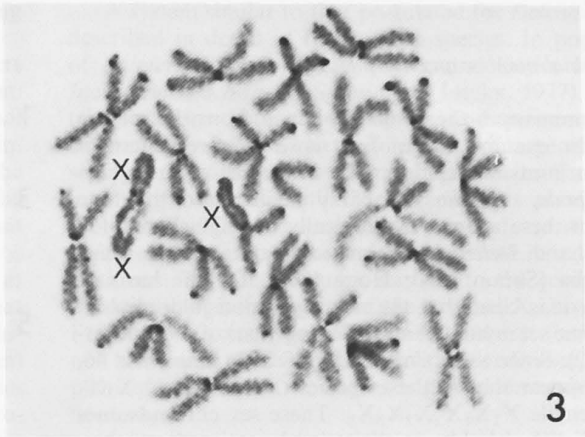
The *Olios* and *Isopoda* data represent more than one species and these are not always easily distinguishable. *t* telocentric, *m* metacentric, *C* chain, *II* bivalent, *I* univalent

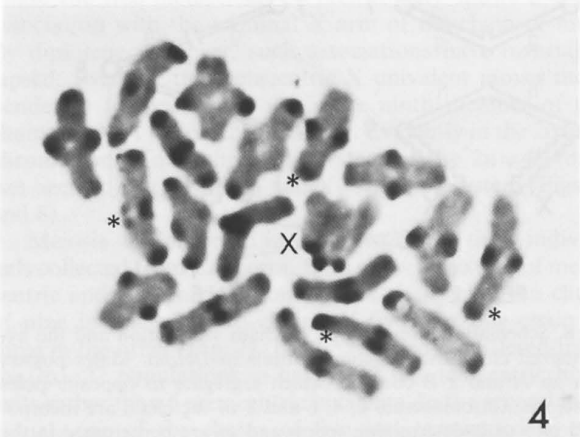
Australia and its associated islands. This is sometimes encountered in colonies consisting of 1 to 3 adult males, up to 6 adult females and successive cohorts of young which may number up to 300 individuals, all in close contact. The young remain in the colony until adulthood.

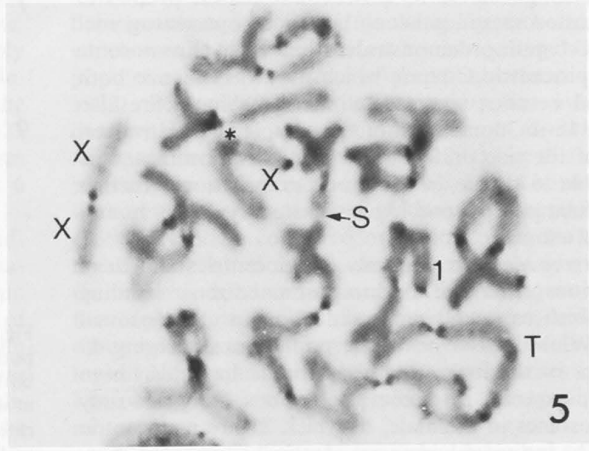
From personal observations I can confirm that in all of the species studied except *D. cancerides*, if two individuals are placed together in a vial, one is quickly killed and usually eaten. However, a large number of individuals from within a colony of *Delena* can be housed together and prey-sharing is often observed. Even so, animals from another colony introduced into the vial are killed and eaten.

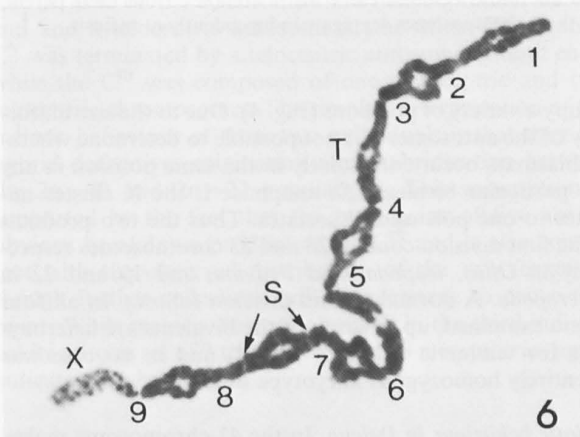
Collection. Specimens of the huntsman genera *Olios, Isopoda, Pediana* and *Heteropoda*, and of *Delena cancerides*, were collected from under the bark of *Eucalyptus, Callitris* and *Acacia* trees, and from the walls of houses, throughout eastern Australia. Collection sites for *Delena* are shown in Figure 1.

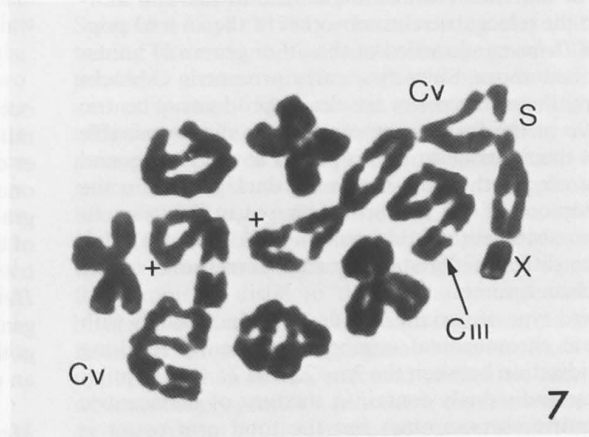
Cytological techniques. Testes of adult and subadult males were used to study male meiosis and spermatogonial mitosis. Mitoses from both males and females were obtained from 2-week-old embryos. The tissue was dissected into insect saline at room temperature and subjected to a hypotonic treatment by the addition of an equal volume of distilled water, and left to stand for 5 to 10 min. At this point the 50/50 insect saline/distilled water mixture was replaced with methanol:acetic acid (3:1) fixative which was repeatedly changed at half hourly intervals. After 3–5 h the tissue was placed on a clean microscope slide in 3 drops of 60% acetic acid and 'tapped out' with the flat end of a 1/8″ thick brass rod. Slides were dried down on a hotplate at 37° C and stained for 3 min in 5% Giemsa stain in phosphate buffer.











Figs. 2–7. Figures 2, 3 C-banded mitosis. **Figure 2** *Isopoda* sp., embryonic mitosis. **Figure 3** *Delena cancerides* spermatogonial mitosis. Autosomes in spermatogonial mitotic preparations are always characterised by splayed chromatids with a granular appearance. The X chromosomes stain more darkly and their chromatids remain closely aligned. **Figures 4–7** Meiosis. **Figure 4** *Isopoda* sp., C-banded. Bivalents heterozygous for C blocks marked (*). **Figure 5** *Delena cancerides* $C^{IX}+6\,II+1\,I$, C-banded. Note that the terminal X arm of the chain multiple overlaps the extended centromeric region of a neighbouring autosomal bivalent (*). See also Figure 6. **Figure 6** *Delena*, C^{IX}. The individual chain members are numbered successively from 1 to 9, with the X-chromosome forming one arm of chromosome 9. **Figure 7** *Delena cancerides*, derived hybrid. $2\,C^{V}+a\,C^{III}+5\,II$, metacentric X missing from this cell. The two chains-of-five result from the replacement of one metacentric with two telocentrics at the positions marked "+". A similar replacement in one of the free bivalents has produced the chain-of-three. *S* secondary constriction, *T* chiasma between chromosomes 3+4 of the chain. This chiasma is always near terminal

Results

Chromosomal number and morphology

Table 1 summarizes the results obtained from the mitotic and meiotic examination of five representative genera of Australian huntsman spiders. The first three of these (*Pediana, Isopoda,* and *Olios*) probably include more than one species but these are not cytologically distinguishable. On the other hand, *Delena* is recognized as a monotypic genus in Australia (Simon 1880; Hogg 1902). On the basis of these data it is clear that the most common male diploid chromosome set in huntsman spiders consists of 43 telocentrics (Fig. 2). Since the corresponding female number is 46, it would appear that in these species the male is $X_1X_2X_3$ and the female $X_1X_1X_2X_2X_3X_3$. These sex chromosomes are not distinguishable in their condensation properties from the autosomes in either embryonic or spermatogonial mitoses. C-banding demonstrates that each chromosome carries a procentric C-block which may vary in size both within and between species. Telomeric C-blocks are also present in 18 to 30 members of the complement. However, because of the size uniformity of the chromosomes, it is not possible to decide from mitotic preparations whether any particular pair of homologues is heterozygous or homozygous for telomeric blocks.

In *Heteropoda* there are only 41 telocentrics and there is no obvious difference in chromosomal size or banding pattern which can explain the reduction in chromosomal number. While *Delena* has some populations carrying 43 telocentrics in the male, 2 other variants have also been seen in this species. In some populations there are only 22 chromosomes in the male, of which 21 are metacentric (Fig. 3). The individual arms are identical in size and morphology to the telocentric chromosomes of the 2n = 43 populations of *Delena* and indeed of the other genera of huntsmen described above. Since they carry procentric C-blocks in each arm, the metacentrics are clearly products of centric fusion. Two of the fusion metacentrics are distinguishable in terms of their condensation properties at spermatogonial mitosis. In one, both arms stain more darkly while in the second, only one of the two arms behaves in this way. In these arms, sister chromatids remain aligned in parallel. These three differentially staining arms correspond to the three sex chromosomes.

The third type of variant includes male individuals with intermediate chromosomal numbers, apparently resulting from hybridisation between the 2n = 22 and 2n = 43 populations. These individuals contain a mixture of metacentric and telocentric chromosomes but the total arm count is always 43.

Meiotic behaviour

The telocentric species. In the prediplotene stages of meiosis in *Olios, Isopoda, Pediana* and *Heteropoda* the three darkly staining X chromosomes are visible in the nucleus, usually in a group, but occasionally separately. As meiosis progresses, the X chromosomes decondense slightly and lie parallel to each other. At diplotene, one X chromosome appears slightly longer than the other two but all three stain with an intensity equal to that of the autosomes. Twenty bivalents are visible in *Olios, Isopoda* and *Pediana*, and 19 in *Heteropoda*, each possessing a single chiasma which may occupy a variety of positions (Fig. 4). Due to the size uniformity of the autosomes it is not possible to determine whether chiasmata occur consistently in the same position in any one particular bivalent. At anaphase I, the X cluster migrates to one pole and dissociates. Thus the two products of the first division contain 20 and 23 chromosomes respectively in *Olios, Isopoda*, and *Pediana*, and 19 and 22 in *Heteropoda*. A normal second division follows. In all four genera examined, up to seven of the bivalents are heterozygous for telomeric C-blocks (Fig. 4) and in no case was an entirely homozygous karyotype observed.

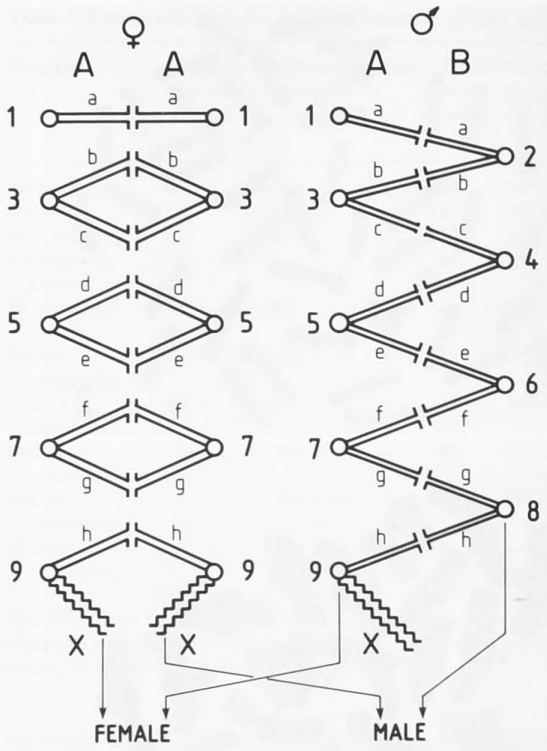


Fig. 8. Schematic representation of chain segregation and the hypothesized chromosomal combinations in *Delena*. Males possess both an A and a B complex which segregate to opposite poles at meiosis. Chromosomes 2, 4, 6 and 8 of the chain are incorporated into male-determining sperm and so are perpetuated in the male line only. Females carry an A complex from each parent, and these chromosomes segregate independently at meiosis

Meiotic behaviour in Delena. In the 43-chromosome males, meiosis is essentially the same as in the all-telocentric species. The only noticeable difference concerns the behaviour of the three sex chromosomes which are less obviously condensed at the early stages of meiosis. The 22-chromosome males, on the other hand, behave like the telocentric species in respect of X-chromosome condensation. Here male meiocytes include an unpaired metacentric X chromosome, six metacentric bivalents all with one chiasma per arm and a chain of nine chromosomes (C^{IX}) composed of eight metacentrics and the single telocentric (Fig. 5). This chain is formed by the pairing of homologous arms and is terminated by the telocentric at one end (referred to as "number one" in the chain) and the X arm of the X-autosome fusion product (number nine in the chain) at the other (Fig. 6).

This structure precludes any prospect of forming a ring multiple.

Each of the paired homologous arms of the members of the chain are again held together by a single chiasma. Chiasma position varies between cells but is always terminal between the third and fourth chromosomes in the chain. Likewise, secondary constrictions are always present in the homologous arms of chromosomes seven and eight in the chain, so that the structure of the chain appears invariant in all individuals which carry it.

The chain adopts an alternate, zig-zag, orientation at first metaphase and second division products confirm that members of the chain segregate alternately. Thus the telocentric (1) and four metacentrics (3, 5, 7 and 9 in the chain) move together to one pole with the other four metacentrics (2, 4, 6 and 8) passing to the other (Fig. 8). At early prophase the free metacentric X occasionally shows a loose association with the terminal X arm of the chain of nine. By diplotene, however, such associations have invariably lapsed. Even so, the metacentric X univalent moves independently to the same pole as the ninth member of the chain, the X-A fusion chromosome. Evidently in the 2n = 22 chromosomal form the X_{1-3} system of the 2n = 43 form has been converted into a neo-X_{1-5}, Y_{1-4} system (Figs. 6 and 8).

Meiosis was observed in only two of the three individuals collected from Canberra. Both show a mixture of metacentric and telocentric chromosomes giving rise to a chain of nine in one and two chains of five plus one chain of three in the other (Fig. 7). The C^{IX} individual differed from the 2n = 22 populations in having 12 free telocentric bivalents rather than 6 metacentric bivalents. In the second individual, one of the chains of five was terminated by the free X arm of the X-chromosome fusion product at one end and a telocentric autosome at the other. The second C^{V} was terminated by a telocentric autosome at both ends, while the C^{III} was composed of one metacentric and two telocentric chromosomes. The simplest interpretation of the meiotic behaviour of these two individuals is that they are hybrid derivatives originating after hybridization between the 2n = 43 and 2n = 22 populations. This assumption is supported by the fact that the two chains of five are an obvious breakdown product of the C^{IX} and is also consistent with the observed distribution of the two forms although further collections will be necessary to prove this point. While meiosis was not observed in the third individual its mitotic set of 3 telocentrics and 20 metacentrics again indicates a hybrid origin.

Discussion

Sex-linked multiple heterozygosity and sociality

Although female cell divisions could not be obtained for the 2n = 22 chain populations of *Delena*, it is clear that the two chromosomal groups which make up the chain, that is 1, 3, 5, 7 and 9 versus 2, 4, 6 and 8 of the chain, are behaving as multiple X and Y chromosomes from a segregational point of view, although it is unlikely that the metacentric autosomes have any role in sex determination. If this assumption is correct, females would be homozygous with a karyotype of 22 metacentric chromosomes and 2 telocentrics (Fig. 8) and would be expected to form 12 bivalents at meiosis.

A system similar to that postulated for *Delena* has been described in detail in two termite species. In populations of *Kalotermes approximatus* (Syren and Luykx 1981) and *Incisitermes schwarzi* (Syren and Luykx 1977) multiple translocations between autosomes and presumptive sex chromosomes have led to the formation of large, alternately segregating translocation chains at male meiosis, maximally involving over half of the 33 chromosomes present in the diploid set. The rearrangements involved are not centric fusions and the breakpoints have occurred in a variety of positions. This, it is argued, has led to the complete loss of morphological integrity of the original X chromosome. Females are homozygous for the X complex, forming normal bivalents at meiosis. As postulated for *Delena*, the complementary derived Y complex is passed through the male line only.

It has been argued that the situation in *Oenothera* has been maintained because it 'locks up' genic heterozygosity which would otherwise be lost by inbreeding, so that the populations exist in a state of perpetual heterosis (Cleland 1972). Permanent genic heterozygosity has indeed been demonstrated in some populations of *Oenothera* and *Isotoma* (Levin 1975; James et al. 1983) but this argument is clearly not sufficient to explain sex-linked heterozygosity because females are permanently homozygous and any heterotic advantage conferred on males would be lost to females. Differential sexual selection could also explain both the origin and the maintenance of the *Delena* situation but this explanation can be discounted because, unlike many other spider species, there is no obvious difference in ecological behaviour or lifestyle between the sexes in *Delena*. Morphological sexual dimorphism is also comparatively minor.

A more appealing argument has been put forward by Syren and Luykx (1981), who have suggested that by maintaining chromosomes in master linkage groups this system produces greater genetic similarity both between parents and offspring of like sex and, more importantly, between offspring of like sex. This is because, in respect of the chromosomes involved in the chain, offspring have only three genomes contributing to their genetic complement – the two of the female parent and the X or Y complex of the male (Fig. 8). This is of course, tempered to some extent by two facts: firstly, genetic material is exchanged between the complexes distal to the chiasmata and secondly, the bivalents not included in the chain segregate independently. Even this independent segregation however, leads to greater uniformity than in the ancestral telocentric race, because, whereas the progenitor's telocentric chromosomes segregate as independent entities, in the fusion metacentrics, recombination of genes situated on either side of the centromere but proximal to the chiasma is precluded. Haplodiploidy in hymenopterans produces a similar effect to the complex segregation in *Delena*, but with greater uniformity in offspring, because no recombination can take place within the male haploid genome and the workers, which constitute the majority of the hive population, are all of one sex. For a social organism dependent on sibling recognition and behavioural and pheromonal communication, the secondary acquisition of enhanced genetic uniformity could be of survival value, though it is appreciated that social species do exist in which neither haplodiploidy nor complex hybridity are involved. This argument is supported by the fact that a large number of species of social termites show some

degree of sex-linked heterozygosity (Vincke and Tilquin 1978), which is otherwise rare in animals.

Although tolerance of young and even maternal care are common in spiders, this phase is generally shortlived and those young which do not disperse are eventually eaten by the mother. The extended, apparently indefinite tolerance phase which characterises *Delena* has not been described in other huntsman spiders and similar behaviour occurs in only 33 of the 30,000 known spider species (Burgess 1978). These 33 are all snare- or web-building rather than hunting species, however, and a web-living lifestyle appears more conducive to the evolution of colonial behaviour. *Delena* also differs from these species in that only spiders from the same colony are tolerated. In this respect, kin selection may be a major factor in the evolution and maintenance of communal behaviour in *Delena*.

Although colony formation is common in *Delena* it is certainly not obligatory and no caste differentiation of the kind present in the social insects is involved. These latter, however, have very different requirements for survival and it is possible that the lifestyle of an opportunistic predator does not lend itself to any partitioning of labour. Sheer weight of numbers is probably a more efficient method of capturing prey, and tolerance of physical contact and prey sharing between community members may be all that is necessary for this. It should be recognised, however, that although these behavioural changes may seem minor in other groups, social interaction of this kind is very rare in spiders and previously unknown in hunting spiders. It is suggested that in *Delena*, the sex-linked fusion heterozygosity has been selected for and maintained as a direct result of the uniformity it confers on the social groupings which are peculiar to this huntsman species.

Translocation/fusion heterozygosity: permanence versus polymorphism

One of the consistent differences in animals with permanent as opposed to floating translocation or fusion heterozygosity is the involvement of the sex chromosome system in the former and its absence in the latter. In European populations of *Mus musculus* many autosomal centric fusions have been fixed in the homozygous state (Capanna et al. 1976). Indeed, every autosome is known to have been involved in a fusion event in at least one and often many populations. Fusion heterozygosity occurs along hybrid zones between parapatric populations but no cases of fixed heterozygosity have been described. Similarly, many populations of the cockroach *Periplaneta americana* carry high levels of floating polymorphism for chromosomal rearrangements, resulting in rings or chains of up to eight chromosomes at meiosis (Lewis and John 1957; John and Qurashi 1964). Here again, despite extensive rearrangement of the karyotype, no translocations involving the sex chromosome have been recorded. In populations of translocation or fusion heterozygotes in which sex chromosomes are involved, on the other hand, structural heterozygosity is always fixed, at least in some parts of the species range. The simplest examples of this involve 1 or 2 translocations, as observed in 22 termite species (Syren and Luykx 1977; Vincke and Tilquin 1978; Syren and Luykx 1981), the jumping spider *Pellenes* (Maddison 1982) and the rodent *Deltamys kempi* (Sbalqueiro et al. 1984).

All of these observations point to the fact that the involvement of sex chromosomes is a potent factor in the production of permanent structural heterozygosity in animals. One reason for this is that once a translocation between an autosome and a sex chromosome has occurred the autosome becomes rigidly tied to the sex chromosome by the rules of segregation and balanced disjunction which govern sex determination. Thus the original autosomal pair becomes in effect a pair of neo-sex chromosomes and breakdown of the acquired structural heterozygosity can only occur either through a precise reversal of the translocation or a breakdown of the sex-determining system. A sex chromosome-autosome rearrangement, then, assures preservation of the rearrangement in a heterozygous state in the heterogametic sex. Complex heterozygosity could be maintained without sex-chromosome involvement through a system of balanced lethals as in *Oenothera* and *Isotoma* but this comes a poor second in terms of fertility and fecundity due to the resultant high proportion of inviable gametes or zygotes inevitably produced.

The origin of fusion heterozygosity in Delena

The origin of permanent structural heterozygosity has been the subject of considerable controversy in the past and it is probable that no single mechanism is responsible for its occurrence in all of the cases observed. Two general theories have been proposed for the origin of translocation heterozygosity in the subgenus *Oenothera*. Cleland (1972) argued that multiple formation occurred suddenly, as the result of hybridization between species homozygous for different translocations. He also emphasised that different self-incompatibility alleles carried by the parental types then immediately provided the balanced lethals necessary for maintaining structural heterozygosity. That is, the alleles which prevented the parentals from selfing are now self-incompatibility alleles only for the complex to which they belong and so effectively prevent homozygosity for the complex in the offspring of the hybrid. Cleland's argument for a hybrid origin is supported by the fact that the same complex may be present in more than one structurally heterozygous species, suggesting that the same ancestral homozygous species has been involved in the parentage of these distinct, derived species.

Darlington (1931) on the other hand, argued that the multiples were produced initially by a reciprocal translocation between two non-homologues and were built up in a stepwise fashion by the incorporation of free chromosomes into the ring via additional reciprocal translocations. This was facilitated by inbreeding "such as we expect on the edge of an advancing species" (Darlington 1973) and maintained for its utility in countering the trend toward genic homozygosity produced by that inbreeding. Darlington believed that the failure to find any interchanged homozygotes provided strong evidence against Cleland's hypothesis of a hybrid origin. The fact that some species share identical complexes is, he argued, the result of secondary hybridization after the establishment of complex structural heterozygosity in the parental species.

Some degree of outbreeding was necessary under Cleland's model for the initial hybridisation events but the model requires that the maintenance of the ring structure results from the inbreeding habit of these species. In this respect Darlington's model is more parsimonious in the sense that only inbreeding is required both for the evolution

and maintenance of translocation heterozygosity and this type of breeding system is well known to characterise many of the *Oenothera* species. Both hypotheses hold that selection was a driving force behind the origin of this chromosomal system which maintains genic heterozygosity under conditions of inbreeding that would otherwise lead to high levels of homozygosity.

Whatever the origin of translocation heterozygosity in *Oenothera*, it would appear that in the termite *K. approximatus* the interchange multiples have been built up by successive translocations between free autosomes and the chromosomes already involved in a simple translocation system, since chain size increases in a northerly cline (Syren and Luykx 1981).

In *Delena*, however, the features of the karyotype and the pattern of population distribution suggest that chain formation was rapid and could not have involved fixed intermediate-length chains. The fact that the one chromosomally intermediate group sampled to date (Canberra) contains no fixed intermediate-length chains, together with the geographic location of this population strongly suggest a hybrid origin for these individuals, resulting from a point or zone of contact between the $2n = 43$ and $2n = 22$ populations. There is certainly no indication of a fixed intermediate-length chain even in these putative hybrids, which have either a maximum-length chain or else obvious breakdown products of such a chain. Moreover, the formation of permanent higher order chains is precluded by the fact that the free-floating bivalents have themselves undergone fusions. Any increase in chain length would require a fission of one of these chromosomes and subsequent refusion to the telocentric which terminates the chain.

While it may be difficult to accept that completely complementary fusion complexes could have arisen at one stroke, the available data are best interpreted by assuming that the C^{IX} forms arose as the result of hybridisation between populations fixed for different fusion products. The chain then originated directly from the resultant heterozygosity for the different fusion products of the parental karyotypes. The floating bivalents may have originally been heterozygous for fusions and then drifted to a homozygous condition due to the lack of association with the sex chromosomes. The hybrid population, possessing a competitive advantage over the homozygous populations, spread outward from its point of origin to achieve its present distribution and in so doing extinguished the parental types.

One weakness of this model is that it relies on the evolution of two populations both carrying numerous centric fusions, which rarely occur even as heterozygotes in spider populations. Indeed the only example of such a centric fusion is in the jumping spider *Pellenes* (Maddison 1982). In the normally telocentric species *Mus musculus*, however, many isolated populations have been described which possess one or many centric fusions in the homozygous state. These populations are almost exclusively found on small islands or in isolated valleys and fixation is generally held to be the result of geographic isolation, founder effects and associated inbreeding (Nash et al. 1983; Gropp et al. 1972). Larson et al. (1984) argue that social structure may also have played a part in the fixation of these arrangements by inducing non-random mating patterns. Similarly, as a tree-dwelling species whose only method of dispersal is ambulation, *Delena* populations are particularly prone to fragmentation, isolation and founder effects. Bushfires are common throughout the species distribution and, as a consequence, must produce frequent and drastic population bottlenecks. Similarly, the clearing of forests for grasslands has provided isolated habitats and effective barriers to dispersal as have the many permanent rivers in the region. Inbreeding imposed by social structure may also favour rapid fixation of chromosomal rearrangements.

The parental populations envisaged need not have been very large and may have been composed of only a very few individuals or colonies, possibly belonging to a larger population with a predisposition for forming random centric fusions, as has been proposed for the rearranged *Mus* populations (Nash et al. 1983; Gropp et al. 1972). If such a situation were coincidental with a post-fire recolonisation, the establishment of a hybrid population may have been very rapid, with little chance of dilution by parental forms and without the necessity for any large, initial competitive advantage. Further discussion relating to possible variations to the basic model is of heuristic value only, however, because the wide distribution of the hybrid form renders it unlikely that the parental types are still extant.

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Complex Sex-linked Translocation Heterozygosity: Its Genetics and Biological Significance

David M. Rowell

Chromosomal translocations (interchanges and fusions) can alter linkage and random assortment when present in the heterozygous condition. In certain circumstances, this heterozygosity can be maintained through an indefinite number of generations. Sex-linked translocation heterozygosity, in which males are perpetually heterozygous for translocations while females are perpetually homozygous, is disproportionately frequent in social and eusocial arthropod species. This article surveys the reported instances of sex-linked translocation heterozygosity, and reviews recent explanations for the observed correlation with social behaviour.

Genes carried on the same chromosome are linked in inheritance. The degree of linkage (the degree to which they are inherited together) normally depends upon their distance apart on the chromosome: genes which are close together rarely undergo recombination whereas genes which are far apart are regularly recombined at meiosis. In the absence of recombination (achiasmatic meiosis), or with restricted recombination, linkage can be virtually absolute. In most organisms, however, linkage is only partial; chiasma positions may vary, and genes carried on different chromosomes generally assort independently of one another at meiosis unless there is non-random segregation of specific chromosomes.

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Two kinds of chromosomal translocation – interchanges and fusions – may alter both linkage and random assortment when present in the heterozygous condition. Such rearrangements extend linkage beyond the limits of a single chromosome, and require preferential segregation for the production of genetically balanced gametes (Fig. 1).

In this example, the chromosomal linkage arises from heterozygosity for the chromosomal translocation. Consequently, this linkage will not be maintained in the next generation if one of the gametes fuses with another of the same type, producing a zygote homozygous for either the translocated or untranslocated chromosomes. If two conditions are met, however, it is possible for the heterozygosity (and hence linkage) to be maintained in future generations *ad infinitum*. Firstly, there must be alternate segregation (Fig. 1); secondly, the gametes must form zygotes only with gametes carrying the opposite translocation type.

Permanent translocation heterozygosity in plants

Two classic examples of chromosomal linkage via permanent translocation heterozygosity are found in the plant genera *Oenothera* ($2n=14$) and *Isotoma* ($2n=14$)[1,2]; in some populations, all of the chromosome pairs have been involved in translocations.

Meiotic behaviour in these species ensures that unbalanced gametes are not generated: rather than forming independent bivalents, meiotic pairing leads to the formation of large ring-shaped complexes on which the translocated chromosomes always appear in the same order. Segregation of these chromosomes at meiosis is thus no longer independent. Rather, every alternate chromosome in the ring migrates to the same spindle pole during the first meiotic division, the remaining chromosomes moving to the opposite pole. Thus, despite the fact that each chromosome is a discrete physical entity, every gamete invariably carries one of two groups of chromosomes. This chromosomal linkage is maintained

through generations by additional mechanisms that ensure perpetual heterozygosity for the translocations. These include balanced lethal alleles, whereby neither chromosome group can produce viable zygotes in the homozygous state, and gametic lethals, which determine that pollen can only fertilize ova containing the opposite chromosome type.

The origin of translocation heterozygosity in these genera may have involved an initial hybridization event between normal bivalent-forming populations homozygous for different sets of translocations, resulting in heterozygous populations[1]; alternatively, the incorporation of chromosomes into the ring may have been effected in a stepwise fashion by the fixation of new translocations in the heterozygous state, perhaps accelerated by founder effects[2–4]. These two mechanisms are not, of course, mutually exclusive.

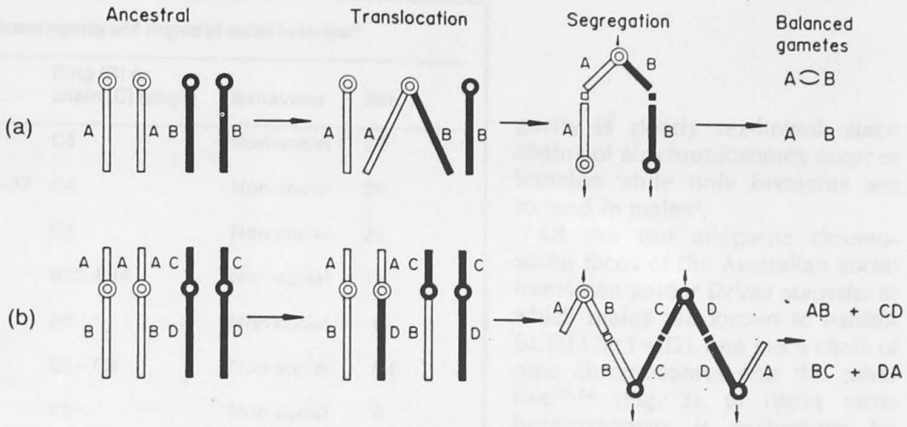

Fig. 1. Chromosomal translocations can lead to higher order gene linkage beyond the limits of a single chromosome. In (a), a single chromosomal fusion has occurred, and meiotic pairing results in the formation of a trivalent. If the gametes produced are to be balanced, and hence viable, they must possess only one copy of each homologous chromosome arm. Thus segregation at division I of meiosis must be 'alternate', as shown, and only two kinds of gametes will result – one carrying the AB fusion product, and the other with the two unfused A and B chromosomes. Consequently, these two unfused chromosomes are effectively linked, since they always occur together in the gametes.

A reciprocal translocation or interchange, as in (b), will lead to the formation of a ring-shaped quadrivalent at meiosis (in this example the ring has been opened out for convenience). Again, viable gametes can only result if alternate segregation occurs, as shown. Thus chromosomes AB and CD are linked, as are BC and DA.

In both examples, the genetic material on these chromosomes will be linked to a greater or lesser degree depending on the number and position of chiasmata. If there is only a single terminal chiasma on each chromosome arm as shown here, gene linkage will be complete.

Permanent sex-linked translocation heterozygosity in animals

Permanent translocation heterozygosity of the kind found in *Oenothera* and *Isotoma* has not been reported in animals. However, translocations involving the sex chromosomes can result in a similar phenomenon, restricted to the heterogametic (and hence usually male) sex.

If, in a male, autosomal material is translocated onto an X chromosome, giving rise to a 'neo-X' product, the homologue of that autosome can be regarded as a 'neo-Y' chromosome, as it must segregate from the neo-X chromosome at the first meiotic division if balanced gametes are to be produced. Consequently sperm will be of two kinds, one kind carrying the neo-X (X–autosome translocation product), and the other carrying the neo-Y (autosomal homologue) which will appear only in male offspring. Conversely, if a translocation occurs between an autosome and a Y or neo-Y chromosome, its non-translocated homologue will move with the X chromosome and so become, from a segregational point of view, an additional neo-X chromosome. At meiosis, the pairing of neo-X and neo-Y chromosomes gives rise to translocation rings or chains (Fig. 2).

Under a translocation system of this kind, males will be perpetually heterozygous for the translocations since they are heterozygous for their sex chromosomes. Females, on the other hand, will be perpetually homozygous for the translocations involved since they are homozygous for their sex chromosomes. These neo sex chromosomes generally differ from the original sex chromosomes in that crossing over still occurs, so there is some degree of recombination between them. Moreover, they generally continue to behave as autosomes in terms of their contraction and staining characteristics at meiosis.

Simple sex-linked translocation heterozygosity (SLTH) of this kind, involving only three or four chromosomes, has been reported in males of a number of species (Table 1). The small rings and chains have generally been produced by the fixation of only one or two rearrangements. In SLTH, a chain rather than a ring of chromosomes is often formed at meiosis, but the alternate meiotic segregation which characterizes *Oenothera* and *Isotoma* still applies. Complex SLTH resulting in long chromosome chains (Fig. 2) is much rarer.

In 1954, Ogawa described a sex-linked translocation chain in males of the centipede *Otocryptops sexspinosus* ($2n=15$), consisting of nine chromosomes[5], five of which appear in males only. The other four behave as X chromosomes and are homozygous in the female. Thus this system acts as an X_{1-4},

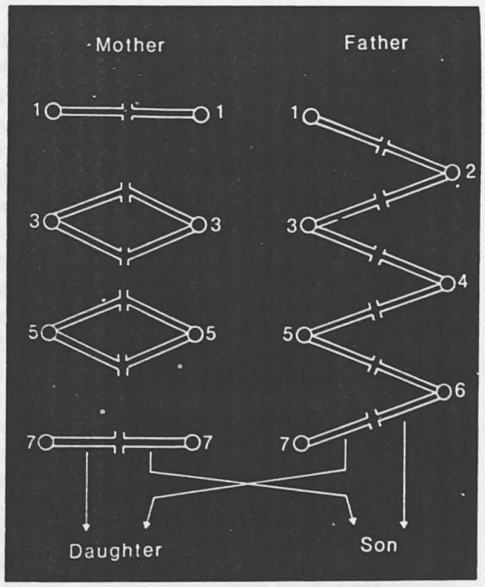

Fig. 2. Schematic representation of a sex-linked translocation chain involving seven chromosomes. The chromosomes involved constitute two groups, analogous to sex chromosomes. The group consisting of chromosomes 1, 3, 5 and 7 behaves as an X chromosome: it is homozygous in the female and carried by all eggs. The male is heterozygous for the two groups (1, 3, 5, 7 versus 2, 4, 6), and so meiotic pairing results in a translocation chain. Chromosomes 1, 3, 5, and 7 are incorporated into female-determining sperm, while 2, 4 and 6 behave as Y chromosomes and migrate together to form male-determining sperm. Thus the sex-determining mechanism maintains the translocations in the homozygous state in females and the heterozygous state in males.

Table 1. Sex-linked translocation heterozygosity and degree of social behaviour[a]

Species	2n	Ring (R) or chain (C) length	Behaviour	Ref.
Cyrsylus volkameriae (flea-beetle)	30	C4	Non-social	27
Nosopsyllus fasciatus (flea)	20–27	C4	Non-social	28
Austroagalloides sp. (leaf hopper)	18	C4	Non-social	29
Mesocyclops edax (copepod)	14	R12, R14	Non-social	12
Diaptomus castor (copepod)	34	R6	Non-social	6
Otocryptops sexspinosus (or *O. sexguttatus*) (centipede)	15	C5 – C9	Non-social	5,6
O. rubiginosus (centipede)	25	C5	Non-social	6
Delena cancerides (huntsman spider)	22	C5, C9	Social	13,14
Isoptera, 24 spp (termites)	32–63	R4 – R18	Eusocial	9,10,25

[a]Only rings or chains of four or more chromosomes have been included, since this requires the fixation of more than one fusion, or a reciprocal rather than a single translocation.

Y_{1-5} sex-determining mechanism. Smaller translocation chains have been discovered in other populations of this species, indicating that the larger chain was probably built up progressively by the gradual incorporation of translocations involving the ancestral X and Y chromosomes or neo-X and neo-Y chromosomes[6]. Another member of the genus, O. *rubiginosus*, also carries chains.

In the termite *Kalotermes approximatus* ($2n=32,33$) translocation chains have also been built up by the successive fixation of novel translocations[7,8]. The presumed ancestral race from southern Florida carries a chain of 11 chromosomes, while populations to the north have chains of 13–17 chromosomes, the number usually constant within populations.

Geographical variation in chain or ring size also occurs in the related termite *Incisitermes schwarzi* ($2n=32$), where from 11 chromosomes (Mexico) to 18 (Jamaica) may be involved[9,10]. The latter represents the longest translocation complex found in any plant or animal so far[10]. Enzyme electrophoresis of a ring-of-14 race of this species showed sex linkage for two loci[11]. At both loci, only one of the two or three alleles present at the given locus is found on the Y group of chromosomes, while all of the alleles may occur on the X complex. Hence males are usually heterozygous for the Y-linked allele and one of the X-linked alleles, while female heterozygosity levels are much lower. This linkage is not complete however, since recombination at meiosis, via chromosomal crossovers, occasionally results in alleles carried on the X complex being transferred to the Y complex and appearing in the homozygous state in males.

The copepod *Mesocyclops edax* ($2n♀=14$) differs from most other species with SLTH in forming a ring complex rather than a chain at meiosis[12]. Moreover, no recombination takes place here because meiosis is achiasmate. This species is also polymorphic, with two different meiotic configurations present in the same population. In 30% of individuals all 14 chromosomes are involved in a translocation ring, but in the rest there is a ring of 12 chromosomes and one free bivalent. Since, from the ring configurations, the two forms cannot be attributed to a single polymorphism for a translocation between one of the bivalent-forming chromosomes and those in the ring[12], the two ring types may represent sympatric, reproductively isolated populations.

Whether the system described in *Mesocyclops edax* is sex-linked or not is also unclear, since only females have been karyotyped. There is evidence to suggest that females are the heterogametic sex in this group[12], which is consistent with sex linkage; but since males have not been studied meiotically, the possibility that this system is maintained by balanced lethals (as in *Oenothera*) rather than sex linkage cannot be ruled out. However, in another copepod species, *Diaptomus castor*, translocation heterozygosity is clearly sex-linked, since chains of six chromosomes occur in females while only bivalents are formed in males[6].

Of the two allopatric chromosome races of the Australian social huntsman spider *Delena cancerides* in which males are known to exhibit SLTH ($2n♂=22$), one has a chain of nine chromosomes and the other five[13,14] (Fig. 3). In these races heterozygosity is exclusively for chromosome fusions rather than interchanges, but the meiotic configurations and segregation patterns are similar to those described above. Additionally, all of the chromosomes not included in the chain multiple have also undergone centric fusions. This reduces their chance of subsequent incorporation into the chain complex via fusion, since a chromosome fusion would be necessary prior to the required fusion. Furthermore, since no races exist with a mixture of fused and unfused chromosomes, a hybrid origin seems likely for these two races, involving parental races carrying different fusions. Electrophoretic evidence also suggests a hybrid origin, as in the chain-of-nine race the Y complex carries only one allele for the enzyme aldolase, and the X complex another.

The biological significance of complex SLTH

Despite its superficial similarities, SLTH is a very different phenomenon from the translocation heterozygosity of *Isotoma* and *Oenothera*, because it is confined to one sex. The chromosomes segregate randomly in the female so that while the Y-linked group of chromosomes remains constant, the X-linked set undergoes considerable reassortment – a unique form of Darlington's 'two track heredity'[15].

The following consequences of SLTH were listed by Syren and Luykx[7]:

(1) restriction of many alleles to males via the Y complex;
(2) maintenance of extensive genic heterozygosity;
(3) increased genetic similarity between like-sex offspring.

Since the Y-linked group of chromosomes never appears in females, males can evolve, to some extent, independently of females. Differential selection on one sex, or

drift, could result in males becoming ecologically, behaviourally and morphologically divergent from females. Furthermore, such divergence may be expected to be very rapid, since the Y-linked group never occurs in the homozygous state, and so no reassortment between separate lineages with a consequent dilution of accumulated changes can occur. Thus, a new mutation in the Y complex of a single individual male is essentially fixed (albeit in the heterozygous state) in all of his future offspring.

Surprisingly, increased sexual divergence does not appear to have occurred and, where data on species with this chromosomal system are available, the two sexes are remarkably similar. In *Delena*, males and females are behaviourally and morphologically indistinguishable until shortly before maturity, and in *Incisitermes schwarzi* there is no preferential representation of either sex in any of the castes[16,17]. Colonies involved in nymph and alate formation do show an excess of males in all castes, but this is a function of an overall temporary bias in the sex ratio caused by the earlier development of female workers into nymphs and alates, and their earlier dispersal. Castes made up of only one sex have been reported in *Nasutitermes exitiosis* and some other higher termite species[18], but the longest chains have been recorded in the lower termites, where this does not occur.

Thus, although there is some electrophoretic evidence for genetic divergence between the sexes, and consequently the potential for phenotypic divergence, there is no evidence that the latter has occurred. Hence, it appears unlikely that selection for this effect has been responsible for the evolution of complex SLTH in the Isoptera or in *Delena*.

That the maintenance of genic heterozygosity has constituted a selective pressure for the development of SLTH also seems doubtful, on the grounds of the similarity which exists between the sexes. Although this chromosomal system has been demonstrated to result in the maintenance of genic heterozygosity in males[11], it can only be achieved at the cost of lowered heterozygosity levels in females, and the apparently identical ecological requirements of the two sexes does not support the argument for differential selection.

From a computer simulation, Charlesworth and Charlesworth[19] arrived at a model in which 'a necessary condition for selection to favour a fusion between an autosome and a sex chromosome is that the alleles at the autosomal locus are maintained by selection at different frequencies in the two sexes'. As mentioned above, in *Delena* and *Incisitermes schwarzi*, which both carry large translocation complexes, there are no marked differences between the sexes that would indicate differential selection on morphological or behavioural grounds. Moreover, many other species of spiders show extreme sexual dimorphism, and yet a literature search shows that nearly 300 spider species have been examined chromosomally and none possess complex SLTH.

However, Charlesworth and Charlesworth's model does depend on the assumptions that the fusions are not themselves associated with any effect on fitness, nor subject to distorted segregation in their favour in heterozygotes[19]. The possibility that SLTH may indeed have a selective value beyond the genotype *per se* has been the subject of discussion[20–24], particularly with regard to social animals, in which an unexpectedly high proportion of species have been found to carry SLTH.

SLTH and social behaviour

From Table I it is clear that a disproportionate number of social, especially eusocial, species carry SLTH; furthermore, the largest translocation complexes occur in euosocial species. Some of the 24 termite species may share this chromosome system through descent, but the fact that the phenomenon appears sporadically in three separate families[25] indicates that it has arisen more than once in the Isoptera.

It has been suggested that social behaviour may facilitate the rapid fixation of chromosomal changes, and inbreeding in social species may increase the chances of fixation of translocation heterozygosity[9], but while many non-social species inbreed, the correlation with social behaviour remains. Furthermore, in some chain-carrying termite species cross-fertilization appears to be favoured[25]. Thus it is worth considering the possibility that this chromosomal system has some adaptive function in social species.

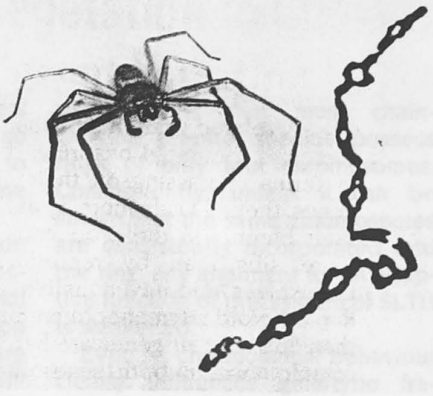

Fig. 3. Male specimen of the social huntsman spider *Delena cancerides* and its meiotic translocation complex consisting of nine chromosomes. Five of these are incorporated into female-determining sperm and the other four into male-determining sperm.

As mentioned above, SLTH can alter the relationships within family groups such that individuals will be more closely related to their like-sex sibs than to siblings of the opposite sex. The extent of the increased relatedness depends on the number of chromosomes involved in the translocation complex and the position of crossing over at meiosis. In the most extreme situation where all of the chromosomes are involved, and crossovers are always distal or absent (as in *Mesocyclops*), relatedness between like-sex offspring would be 75% compared with 50% between parents and offspring. This is because offspring receive a random 50% of their genes from their mother, but *the same* 50% from their heterozygous father – the Y-linked group in the case of male offspring, and the X-linked group in female offspring. Relatedness between opposite-sex offspring would be 25% through the female parent and zero through the male. Although translocation heterozygosity never involves all of the members of the chromosome complement in termites, in two species studied to date a large proportion is involved and the crossovers are generally terminal.

Lacy[20,22] argued that this potential bias in relatedness has played a role in the evolution of eusocial behaviour in the termites, on the same grounds that Hamilton[26] suggested haplodiploidy has been relevant to the evolution of eusociality in the Hymenoptera. That is,

because individuals are more closely related to their sibs than to their own potential offspring, then in terms of passing on their own genes, they will gain more by aiding their parents in the production of more sibs than by reproducing themselves. Moreover, unlike the haplodiploid Hymenoptera, more than 50% of their genes are held in common within both sexes. Both sexes would therefore be expected to participate in worker duties, as is in fact the case. The analogies between these two genetic systems are striking, and Lacy's idea has the attraction that it 'brings under one roof the problem of the evolution of eusociality in two unrelated groups of insects'[16].

Nevertheless Lacy's hypothesis has been criticized on two points:
(1) Although relatedness is enhanced in offspring of the same sex, the corresponding reduction in relatedness between sexes would dictate that workers should preferentially invest in offspring of their own sex. For certain castes, such as soldiers, this is clearly not possible since their labour benefits the colony as a whole[21]. Furthermore, there is no evidence for this idea from data on the spatial distributions of the sexes in a termite colony[16].
(2) Translocation heterozygosity apparently arose in termites *after* the evolution of sterile workers[23,24]. That this system could have been selected to justify the existence of sterile workers already present is clearly illogical.

Furthermore, in *Delena* the chain-carrying populations appear to have displaced the ancestral, homozygous populations, again indicating that this chromosomal system may have an adaptive significance. However, sterile workers are absent in this social species, so any adaptive value attributed to SLTH here must pertain to some other aspect of colonial life.

Another suggested explanation for the apparent selection for translocation heterozygosity in social species is based on the assumption that because same-sex sibs are more closely related, they will share more genes in common and thus vary less in their genotypes[13,24]. Consequently, a greater uniformity would be expected in the genetically determined aspects of behaviour, including reactions to different pheromone levels and pheromone release in response to external factors. Clearly, uniformity in morphology is not necessarily of great importance since termite colonies owe their efficiency to their range of morphological castes, but some uniformity in communication mechanisms within and between castes, such as pheromone release, may well be advantageous. In a colony whose cohesiveness and competitiveness relies on the reaction of its members to pheromonal stimuli and behavioural cues, increased uniformity in behaviour and reactions would be expected to result in greater efficiency and ultimately greater reproductive success.

One weakness in this argument is that although SLTH increases uniformity within sexes, genetic disparity between the sexes remains. This would lead to two distinct genetic groups, although there would be relative uniformity within each group, resulting in a colony comprised of two relatively uniform groups rather than the range of types possible in a normal diploid population. A second possible weakness is that most chain-carrying termite species possess rings of only four chromosomes. Consequently, unless it can be shown that the same chromosomes are consistently incorporated into the ring, any argument for an adaptive function of this system of SLTH is weakened.

Even so, chromosomal behaviour clearly influences genotype frequency distributions within and between the sexes. It remains to be established whether its correlation with social behaviour is a casual or causal phenomenon.

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COMMENT

Complex sex-linked translocation heterozygosity and its role in the evolution of social behaviour

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Permanent sex-linked complex translocation heterozygosity is known to occur in at least 24 termite species (Vincke and Tilquin 1978) and its possible function has been the subject of speculation since first described in *Incisitermes schwarzi* (Syren and Luykx 1977). The fact that this specialized genetic system is otherwise rare in animals provides circumstantial evidence that it may somehow be involved in the evolution of eusociality. Lacy (1980) pointed out that complex sex-linked heterozygosity is similar to the haplodiploidy of hymenopterans with respect to the enhanced relatedness it produces within colonies. Thus, offspring of the same parents will be more closely related to their same-sex sibs than to any potential offspring of their own. This led Lacy to argue that "a tendency to invest resources in same-sex sibs rather than in offspring would therefore increase inclusive fitness." Leinaas (1983) on the other hand pointed out that sex-specific investment is impossible for some of the nonreproductive castes such as soldiers, which, by guarding the nest and the workers' trails "automatically guard all their sibs equally." Similarly, by nursing the royal couple, workers cannot show preference for their own sex. Moreover, while a rebel worker which concentrated on raising only own-sex sibs would be favoured by selection, it would disadvantage the colony as a whole. This highlights the fact that as social organization becomes more complex, individual fitness *per se* becomes less important than the contribution of the individual to the inclusive fitness of the colony as a whole. Thus, Leinaas argued, the asymmetry of relatedness resulting from complex heterozygosity is not relevant and in fact complicates the problem of the evolution of sterile castes.

The recent discovery of complex sex-linked heterozygosity in the social Australian huntsman spider *Delena cancerides* provides further evidence that this genetic system may be related in some way to social behaviour (Rowell 1985). However, there are good grounds for concluding that permanent sex-linked translocation heterozygosity has not been selected for simply to justify the existence of sterile workers.

Delena cancerides often occurs in colonies consisting of 1 to 3 adult (reproductive) females, up to 6 adult males, and as many as 300 juveniles representing successive cohorts from hatching to subadults. The main limiting factor on colony size appears to be the availability of sufficient space either in the form of large pieces of bark still attached to native trees, under which the colony is able to shelter, or in similar habitats, such as woodpiles. The colony members rest in contact with one another and have been repeatedly observed in the laboratory sharing prey. Both of the activities are very unusual for spiders, which are often aggressively cannibalistic. However, mutual tolerance only occurs within colonies, and outsiders introduced into a colony are quickly killed and eaten, indicating that a precise recognition system exists.

Although hatchlings may take two or three seasons to reach sexual maturity, there is no evidence that any persist beyond this point as nonreproductives, or that any morphological variation occurs of the kind found in the social insect castes. That juveniles vastly outnumber adults suggests that few actually reach sexual maturity, however, and in this respect it can be argued that juveniles approximate to the state of termite workers, which in some species such as *Mastotermes darwinien-*

sis and *Incisitermes schwarzi* potentially can, and often do, become full reproductives (Watson and Sewell 1981; Watson and Abbey 1985; Luykx 1985). That is, by helping to protect the colony and bring down large prey, they indirectly aid their parents and so ensure that genes held in common are passed on even if they themselves do not reach maturity.

In some populations of *D. cancerides* normal bivalents are formed at male meiosis, but in others, 10 of the 22 chromosomes of the male diploid complement are sex-linked, 9 via a complex translocation chain and the other through directed segregation relative to this chain, the entire system behaving as a multiple XY complex (Rowell 1985). While these chromosomally heterozygous populations clearly arose from, and have in turn displaced the bivalent formers, both types of populations are equally capable of forming colonies.

Social behaviour, then, must have predated complex heterozygosity in this species, though the success of the heterozygotes in displacing their progenitors indicates some selective advantage to the heterozygous populations rather than heterozygosity being passively accumulated as a result of some component of sociality.

In the case of termites, Lacy (1980) argued that since translocation complexes appear in the morphologically less specialized families, they represent the "primative termite genome." This argument is open to objection on four grounds. (*i*) There is no necessary relationship between chromosome change and morphological change. That is, there is no reason to expect primitive morphology to be associated with a "primitive" karyotype. For further discussion on this point see John (1981, p. 29–). (*ii*)The presumed "primitive" family Kalotermitidae in fact includes bivalent-forming species, species with translocation complexes, and species showing both forms, as does the more derived Termitidae (Vincke and Tilquin 1978; John 1983). and *Mastotermes darwiniensis*, which is morphologically most similar to the ancestral termite (Luykx 1983), forms normal bivalents at meiosis (D. G. Bedo, personal communication). Hence there is no obvious link between the "primitive termite genome" and the occurrence of translocation heterozygosity. Furthermore, if a "primitive" morphology reflected a "primitive" karyotype as Lacy implies, then, with translocation heterozygosity arising in the early stages of termite evolution on account of its selective advantage, longer rather than shorter chains would be expected in the more recently derived families. This, however, is not the case (Vincke and Tilquin 1978; John 1983). (*iii*) Fixed complex translocation heterozygosity, whenever it appears in plant or animal populations, is clearly a derived condition. The prospect of sex-linked translocation heterozygosity secondarily reverting to bivalent formation would require either numerous exact reverse translocations, which is extremely unlikely, or else missegregation, which would inevitably lead to inviability. (*iv*) There seems little doubt that translocation heterozygosity in the termites must have arisen more than once since it is represented in termites in at least three continents (Africa, North America, and Europe) and in at least three families.

That translocation heterozygosity has arisen and been positively selected for on more than one occasion, for some advantage it confers on a species that has *already* evolved social behaviour is a more plausible argument, particularly when its occurrence in *D. cancerides* is taken into account. Therefore, it is suggested that while complex heterozygosity confers a selective advantage on some social organisms, the condition is not obligatory for the evolution of sociality. In the case of *D. cancerides*, cooperation in hunting and colony protection may more than compensate for any possible disadvantages of colonial life. Indeed, improved foraging and protection may well have been involved in the initial stages of evolution of sociality in both hymenopterans and termites. In termites, sociality may also ensure reinoculation with symbiotic protozoans following ecdysis (Lacy 1980).

In the case of the evolution of sterile castes, other possiblities can be suggested which do not rely on the enhanced relatedness arising from haplodiploidy or sex-linked translocation heterozygosity. For example, if by supporting its parents a bivalent-forming diploid organism can help them produce more offspring than it can provide, through its own reproduction, it would clearly be advantageous for it to remain with its parents, since it is equally related in a genetic sense to both its sibs and its own offspring. If the parents tend to produce offspring of low fertility, or if for environmental reasons the fertility of offspring produced by the parents is low, as appears to be the case in *D. cancerides*, then the probability of passing on parental genes to subsequent generations will be increased through the altruism of the offspring. If the chance of an individual reproducing is sufficiently low and the genetic advantage to be gained by altruism is sufficiently high, then it may be to an individual's best advantage to completely forego reproduction itself, and instead invest all its resources in aiding the reproduction of its parents and their offspring, since this maximizes the probability that genes held in common between reproductives and nonreproductives are transferred to subsequent generations. Similarly, if by aiding its sibs an individual can produce more or competitively superior nieces and nephews compared with its own grandchildren, then the same argument applies. In some termite species sibling queens do indeed occur, often extensively (Thorne 1982; Watson and Abbey 1985).

Because sterile workers have evolved in species without haplodiploidy or complex heterozygosity (Vincke

and Tilquin 1978), these genetic systems are clearly not necessary prerequisites for the evolution of nonreproductive helpers, and other explanations will need to be sought for their function.

One effect that both haplodiploidy and sex-linked translocation heterozygosity have in common is to increase genetic uniformity, both between same-sex sibs and between parents and offspring of the same sex. Such unformity could be of particular value in aiding sibs and parents to recognize each other, whether recognition is visual, olfactory, or behavioural. In *D. cancerides* and in termites, where both sexes are present, this uniformity will be essentially bimodal, with comparatively little variation around the two modes. Thus individuals need only be able to recognize two main forms, rather than a wide range of morphological, pheromonal, and behavioural variants which may occur in a diploid bivalent-forming race or species. This could be especially important in species as aggressive as spiders and ants.

Under the hypothesis presented here, haplodiploidy and translocation heterozygosity have had a fundamentally different role in the evolution of sociality, although they both have a similar effect in decreasing genetic variation in family groups. Haplodiploidy represents a fortuitous preadaptation for social behaviour and has undoubtedly played a major role in the success of the Hymenoptra as social organisms. Sex-linked complex translocation heterozygosity, on the other hand, is a complicated chromosomal phenomenon which can only be selected for once a degree of social behaviour has already been realized. As such, it may also represent a preadaptation for the evolution of sterile castes, but this is a secondary not a primary role.

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